DESIGN AND DEVELOPMENT OF COLON SPECIFIC DELIVERY SYSTEMS OF ACECLOFENAC TABLET

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In

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By

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MAY 2012

CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN AND DEVELOPMENT OF COLON SPECIFIC DELIVERY SYSTEMS OF ACECLOFENAC TABLET"** is a bonafide and genuine research work carried out at the Department of Pharmaceutics, K.K College of pharmacy by **KARTHIK VADLAMUDI, B.Pharm.,** during the year 2011-2012 under the supervision of **Asst. Prof. Mrs. S.L.LAURA KAVIARASU.,** This dissertation is submitted for partial fulfillment of the requirements for the award of degree of Masters of Pharmacy (Pharmaceutics), by the Tamil Nadu Dr. M.G.R Medical University, Chennai-32.

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Place: CHENNAI *Karthik Vadlamudi*

Date:

LIST OF ABBREVIATIONS USE:

Abs	Absorbance
Conc	Concentration
CDDS	Colon specific drug delivery system
DSC	Differential Scanning Calorimetry
F	Formulation
FT-IR	Fourier-Transform InfraRed
g	Gram(S)
GIT	Gastro intestinal tract
GMP	Good Manufacturing Practice
H	Hours
HCL	Hydrochloric acid
ICH	International Conference on Harmonization
IP	Indian Pharmacopoeia
IBD	Inflammatory bowel disease
min	Minutes
mg	milligram
NSAIDS	Non Steroidal Anti-Inflammatory Drugs
nm	Nanometer
RH	Relative humidity
s	Seconds
SD	Standard deviation
UV	Ultraviolet
USP	United States Pharmacopoeia
w/w	Weight by weight
w/v	Weight by volume
λ_{max}	Lambda max
µg, mcg	Microgram

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INTRODUCTION

Among the various routes of administration, the oral route is considered to be most convenient for the administration of drugs to patients. On oral administration of conventional dosage forms drug normally dissolves in the gastro-intestinal fluids and is absorbed from regions of the gastro-intestinal tract, which depends upon the physicochemical properties of the drug. It has a serious drawback in conditions where localized delivery of the drug in the colon is required or in conditions where a drug needs to be protected from the hostile environment of upper GIT. Dosage forms that deliver drugs in the colon rather than upper GIT has number of advantages.

Oral delivery of drugs in the colon is valuable in the treatment of diseases of colon where by high local concentration can be achieved while minimizing side effects. The colon is attracting of an interest as a site where poorly absorbed drug molecule may have an improved bioavailability. This region of the colon having a somewhat less hostile environment which is with less diversity and intensity of activity than the stomach and small intestine. Additionally, the colon has a long retention time and appears highly responsible to agents that enhance the absorption of poorly absorbed drugs.

The simplest method for targeting of drugs to the colon is to obtain slower release rates or longer release periods by the application of thicker layers of conventional enteric coating or extremely slow releasing matrices. These delayed mechanisms are designed to improve the efficacy of the drug by concentrating the drug molecules, where they are needed most and also minimize the potential side effects and drug instability issues associated with premature release of drug in the upper parts of the Gastrointestinal tract, namely stomach and small intestine. Colon targeted drug delivery would ensures direct treatment at the disease site, lower dosing and less systemic side effects. In addition to restricted therapy, the colon can also be utilized as a portal for the entry of drugs into the systemic circulation. For example, molecules that are degraded or poorly absorbed in the upper gut, such as peptides and proteins, may be better absorbed from the more beign environment of the colon.

There is less free fluid in the colon than in the small intestine and hence, dissolution could be problematic for poorly water-soluble drugs. In such instances, the drug may be need to delivered in a presolubilized form or delivery should be directed to the proximal colon, as a fluid gradient exists in the colon with more free water present in the proximal colon than in

the distal colon. Aside from drug solubility, the stability of the drug in the colonic environment is a further factor that warrants attention. The drug could bind in a nonspecific manner to dietary residues, intestinal secretions, mucus or general faecal matter, thereby reducing the concentration of free drug. Moreover, the resident microflora could also affect colonic performance via degradation of the drug.

HISTORY:

In 1942, Svartz discovered that sulfasalazine; the sulfanilamide prodrug of 5-aminosalicylicacid (5-ASA) is effective in the treatment of rheumatoid arthritis and anti-inflammatory disease. The exact modes by which the drug target itself to the colon was elucidated much latter in 1970 i.e., colon specific azo-reductase splits sulfasalazine causing the release of the active moiety 5- aminosalicylicacid. After the several other azo-bonds containing the compounds designed to locally release 5-aminosalicylicacid were synthesized bensalazine, balsalazide and olsalazine. In1986, Saffron and coworkers described the use of azo containing acrylic polymers to the delivery of protein drugs like insulin to the colon. [1]

ANATOMY AND PHYSIOLOGY OF COLON:

Irrespective of therapy desired for local (colonic) or systemic delivery of drug, the development and aim of the drug delivery to colon remains same[2], that is

- The drug must not absorb from other regions of the gastro intestinal tract (GIT).
- It should only suffer negligible degradation in the small intestine lumen.
- The release of the drug in the colon should be at quantitatively controlled rate and the released drug in the colon should be absorbed from the lumen of the large intestine without any appreciable degradation.

In order to meet these properties, a thorough knowledge of the anatomy and physiology of GIT is required. The GI tract is divided into stomach, small intestine and large intestine. In GIT, the large intestine extending from the ileocecal junction to the anus is divided in to three main parts. These are the colon, the rectum and anal canal.

The entire colon is about 5 feet (150 cm) long, and is divided in to five major segments. Peritoneal folds called as mesentery which is supported by ascending and descending colon.

The right colon consists of the cecum, ascending colon, hepatic flexure and the right half of the transverse colon. The left colon contain the left half of the transverse colon, descending colon, splenic flexure and sigmoid. The rectum is the last anatomic segment before the anus[3]. The human GIT and colon were shown in **Figure1.** and **Figure 2.** respectively.

The colon is a cylindrical tube, made up of four-layers, serosa, muscularis externa, sub mucosa, and mucosa. The colon does not have villi, but in the presence of plicae semilunares (cresentic folds) the intestinal surface of the colon is increased to approximately 1300 cm^2.sub mucosa, and mucosa.

The major function of the colon is creation of suitable environment for the growth of colonic microorganisms, storage reservoir of faecal contents, expulsion of the contents of the colon at an appropriate time and absorption of potassium and water from the lumen[4] . The absorptive capacity is very high, in each about 2000ml of fluid enters the colon through the ileocecal valve from which more than 90% of the fluid is absorbed. On average, it has been estimated that the colon contains only about 220 gm of wet material equivalent to just 35 gm of dry matter. The majority of this dry matter is bacteria. Properties of GI tract were shown in **Table1.**

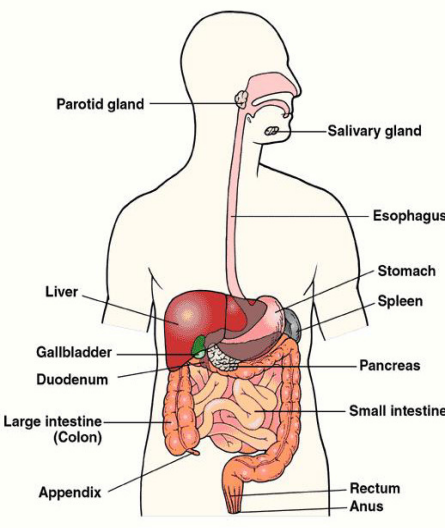


Figure 1: Structure of human GIT

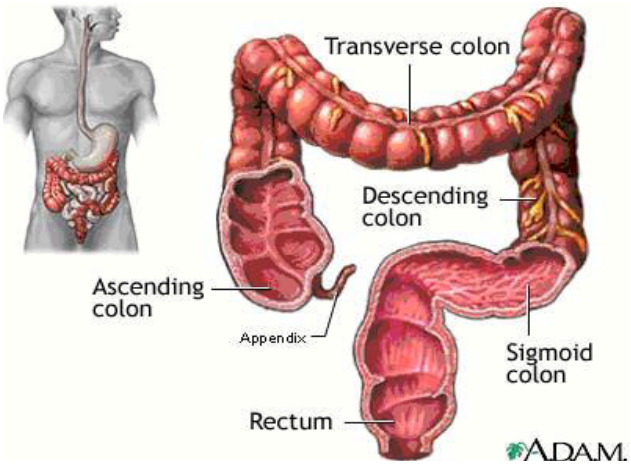


Figure 2: Structure of colon

Table 1: Properties of Gastro Intestinal Tract

Region of GIT	**Property**	**Measured value**
Total GIT	Surface area	2-106 cm^2
Small intestine	Length	
-Duodenum		20-30 cm
-Jejunum		150-250 cm
-Ileum		200-350 cm
Large intestine	Length	
-Cecum		6-7 cm
-Ascending colon		20 cm
-Descending colon		45 cm
-Transverse colon		30 cm

-Sigmoid colon		40 cm
-Rectum		12 cm
-Anal canal		3 cm
Small intestine	Internal diameter	3-4 cm
Large intestine		6 cm
Stomach	pH	Fasted 1.5-2.0, fed 3.0-5.0
Duodenum		5-7
Jejunum		6-7
Ileum		7
Colon		5.5-7
Rectum		7
Colon	Redox potential	- 415
-Right		- 400
-Mid		- 380
-Left		

FACTORS AFFECTING COLON ABSORPTION:

1. Physical properties of drug such as pKa and degree of ionization.
2. Colonic residence time as commanded by GIT motility.
3. Degradation by bacterial enzymes and metabolic products.
4. Local physiological action of drug.
5. Selective and non-selective binding to mucus.
6. Disease state.

TRANSIT THROUGH GIT:

The drug delivery systems first enter into stomach and small intestine via mouth and then reach colon. The nature and pH of gastric secretion and gastric mucus influence the drug release and absorption. In order to reach colon in an intact form, the drug delivery systems should surpass the barriers in the stomach and small intestine. Gastrointestinal transit varies from 1 hr to 3 hrs depending upon the condition (fasting or non-fasting). Normally, the small intestinal transit is not influenced by the physical state, size of the dosage form and presence of food in the stomach. The mean transit time of the dosage form is about 3-4 hrs to reach the ileocecal junction and the time period is consistent. During this period the dosage form is exposed to enzymes present in small intestine. Compared to the other region of GIT, movement of material through the colon is slow. Total time for transit tends to be highly variable and influenced by number of factors such as diet particularly dietary fibre content, mobility, stress, disease condition and drugs. The colonic transit time of a capsule in adult is 20-35 hrs. Improved residence time with subsequent longer transit time and the contact of dosage form with micro flora in colon govern the release and absorption of drug from dosage form as shown in **figure 3.**

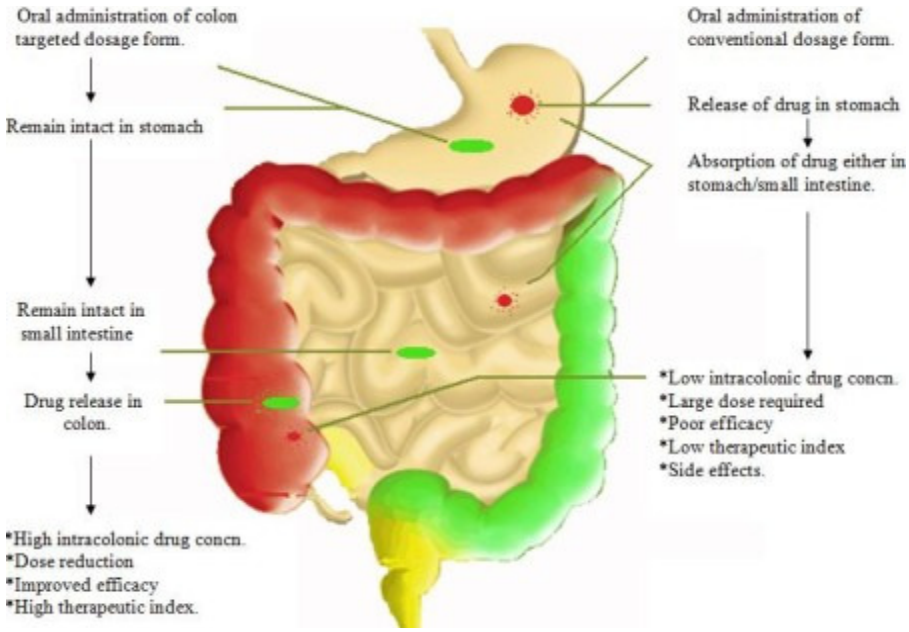


Figure 3: Fate of CDDS tablet after administration

COLONIC MICROFLORA [5]:

The human alimentary canal is highly populated with bacteria and other microflora at both ends, the oral cavity and the colon/rectum. In between these two sites, the GIT is very sparsely populated with microorganisms. Microorganisms of the oral cavity do not affect oral drug delivery systems and as such will not be considered here further. However, gut microflora of the colon have a number of implications in health and the treatment of disease such as IBD. This section presents some background information on gut micro flora as it relates to colonic-based delivery system. Concentration of gut microflora increses considerably in the terminal ileum to reach extraordinarily high levels in the colon . The gut bacteria are capable of catalyzing a wide range of metabolic events. Many colon-specific drug delivery systems on enzymes unique to gut micro flora to release active agents in the colon. However, only two or three enzyme systems have been exploited in this area: azoreductases and glycosidases (including glucuronidase). A large number of polysaccharides are actively hydrolyzed by gut microflora leading to the possibility of using naturally occurring biopolymer as drug carriers. In addition, ethereal sulfate prodrugs or carboxylated prodrugs may be metabolized in the colon to the parent drug leading to local delivery in the colon. There is certainly room for innovative approaches to carry and release drugs in the

colon based on the metabolic capabilities of the colon microflora. Azo-reductases produced by colon play a central role in a number of delivery systems, most notably in catalyzing the release of 5-ASA from a variety of prodrugs. The second class of enzymes used to trigger the release of drugs in the colon is glycosidases (including glucuronidases). The main bacterial groups responsible for beta-glycosidases activity are lactobacilli, bacteroides and bifido bacteria. As with azo-reductase activity, the level of bacterial glycosidase activity in the gastrointestinal tract is associated with the concentration of bacteria in a given region.

STOMACH AND INTESTINAL pH:

Generally, the release and absorption of orally administered drugs are influenced by the GI pH. The gradient in the GIT is not in an increasing order. In stomach the pH is 1.5-2 and 2-6 in fasted and fed conditions respectively. The acidic pH is responsible for the degradation of various pH sensitive drugs and enteric coating may prevent it. In small intestine, the pH increases slightly from 6.6-7.5 and decreases to 6.4 in colon.

Radio-telemetry shows the highest pH level (7.5±0.5) in the terminal ileum. On entry into the colon, the pH drop to 6.4+0.6. The pH in the mid colon is 6.6+0.8 and in the left colon 7.0+0.7. Since there is minimal variation in the pH from ileum to colon, apparently pH dependent polymer drug delivery may not be much selective. However, possible exploitation of pH variation in GIT leads to successful development of various colonspecific drug delivery systems.

GENERAL CONSIDERATIONS FOR DESIGN OF COLONIC FORMULATIONS:

Formulations for colonic delivery are, in general, delayed release dosage forms which may be designed either to provide a 'burst release' or a sustained / prolonged /targeted.

i. Pathology of disease, especially the affected parts of the lower GIT (shown in **figure 4)**

ii. Physicochemical and biopharmaceutical properties of the drug such as solubility, stability and permeability at the intended site of delivery.

iii. The preferred release data of the drug.

Very common physiological factor which is considered in the design of delayed release colonic formulations is pH gradient of the gastrointestinal tract. In normal healthy subjects,

there is a progressive increase in luminal pH from the duodenum (pH is 6.6±0.4) to the end of the ileum (pH is 7.5 ± 0.5), a decrease in the cecum (pH is 6.4 ± 0.6) and then a slow rise from the right to the left colon with a final value of 7.0 ± 0.7. Some reports suggested that alterations in gastrointestinal pH profiles may occur in patients with inflammatory bowel disease, which should be considered in the development of delayed release formulations [6].

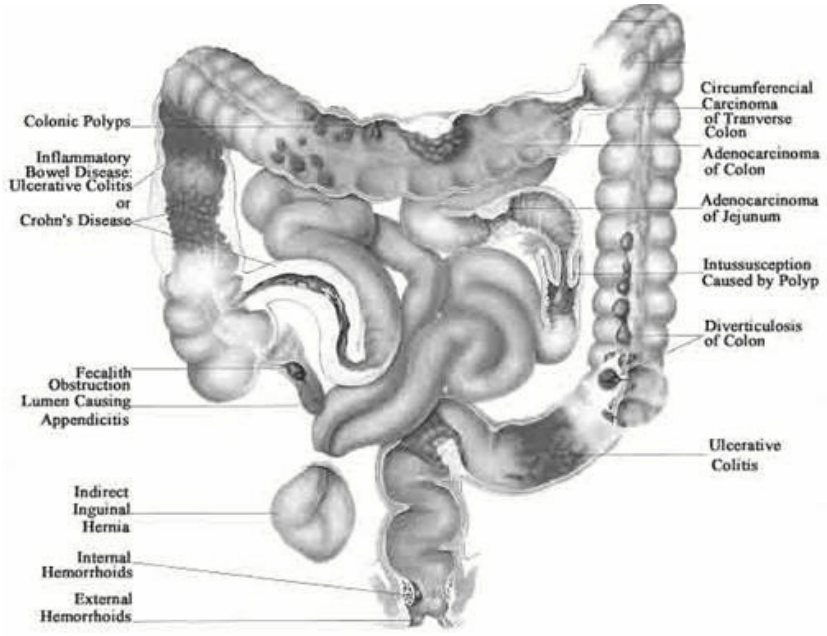


Figure 4: Colonic Diseases

DRUGS SUITABLE FOR CDDS:

The following different categories of drugs are suitable for colon drug delivery.

- Drugs used to treat irritable bowel disease (IBD) require local delivery at drug to colon e.g. Sulfasalazine, Olsalazine, Mesalazine, steroids like Fludrocortisone, Budesonide, Prednisolone and Dexamethasone.

- Drugs to treat colonic cancer require local delivery e.g. 5-Fluorouracil, Doxorubicin, and Methotrexate.

- Protein and peptide drugs - eliminating drug degradation e.g. growth hormones, Calcitonin, Insulin, Interleukin, Interferon and Erythropoietin.

- To treat the infectious diseases like (Amoebiasis & Helminthiasis) which requires site specific delivery e.g. Metronidazole, Mebendazole and Albendazole,

- To treat rheumatoid arthritis (NSAIDS), nocturnal asthma, angina require delay in absorption due to circadian rhythms.

- Drugs which it shows more selective absorption in colon than small intestine due to small extent of paracellular transport e.g. Glibenclamide, Diclofencac, Theophylline, Ibuprofen, Metoprolol, and Oxyprenolol.

LIMITATIONS AND CHALLENGES IN COLON TARGETED DRUG DELIVERY:

1. One challenge in the development of colon-specific drug delivery systems is to establish an appropriate dissolution testing method to evaluate the designed system *in-vitro*. This is due to the rationale after a colon specific drug delivery system is quite diverse.

2. As a site for drug delivery, the colon offers a near neutral pH, reduced digestive enzymatic activity, a long transit time and increased responsiveness to absorption enhancers; The targeting of drugs to the colon is very complicated. Due to its location in the distal part of the alimentary canal, the colon is particularly difficult to access. In addition to that the wide range of pH values and different enzymes present throughout the gastrointestinal tract, through which the dosage form has to travel before reaching the target site, further complicate the reliability and delivery efficiency.

3. Successful delivery through this site also requires the drug to be in solution form before it arrives in the colon or alternatively, it should dissolve in the luminal fluids of the colon, but this can be a limiting factor for poorly soluble drugs as the fluid content in the colon is much lower and it is more viscous than in the upper part of the GI tract.

4. The stability of the drug is also a concern and must be taken into consideration while designing the delivery system. The drug may potentially bind in a nonspecific way to dietary residues, intestinal secretions, mucus or faecal matter.

5. The resident microflora could also affect colonic performance via metabolic degradation of the drug. Lower surface area and relative ‘tightness’ of the tight junctions in the colon can also restrict drug transport across the mucosa and into the

systemic circulation

The literature also suggested that the cytochrome P-450 (3A) class of drug metabolizing enzymes have lower activity in the colonic mucosa. A longer residence time of 3 to 5 days results in elevated plasma levels of the drugs and therefore higher bioavailability in general, but especially for drugs that are substrates for this class of enzyme.

ADVANTAGES:

Colon-specific drug delivery system offers the following therapeutic advantages [1] [7]

- Reducing the adverse effects in the treatment of colonic diseases (ulcerative colitis, colorectal cancer, crohn's disease etc.)
- By producing the environment for peptides and proteins when compared to upper gastrointestinal tract.
- Minimizing extensive first pass metabolism of steroids.
- Preventing the gastric irritation produced by oral administration of NSAIDS.
- Delayed release of drugs to treat angina, asthma and rheumatoid arthritis.
- Drugs which are destroyed by the stomach acid and/or metabolized by pancreatic enzymes are slightly affected in the colon. [8] [9]

DIFFERENT APPROACHES TO TARGET THE COLON

Different approaches to the colon were shown in **Table 2.**

Table 2: Approaches for the development of colon targeted drug delivery

Approach	**Basic feature**
I. Chemical Approaches	
1. Azo conjugates	The drug is conjugated via an azo bond
2. Cyclodextrin conjugates	The drug is conjugated with cyclodextrin
3. Glycosidic conjugates	The drug is conjugated with glycoside
4. Glucuronide conjugate	The drug is conjugated with glucuronate
5. Dextran conjugates	The drug is conjugated with dextran
6. Polypeptide conjugates	The drug is conjugated with polypeptide
7. Polymeric prodrugs	The drug is conjugated with polymer
II. Pharmaceutical Approaches	
1. Coating with polymer	
i. Coating with pH-sensitive polymer	Formulation coated with enteric polymers release drug when pH moves towards alkaline range
ii. Coating with biodegradable polymer	Drug is released following degradation of the polymer due to the action of colonic bacteria
2. Embedding in matrices	
i. Embedding in biodegradable polysaccharides	The embedded drug in polysaccharide matrices is released by swelling and biodegradable action of polysaccharides.
ii. Embedding in pH sensitive matrices	Degradation of pH sensitive polymer in the GIT releases the embedded drug
3. Timed released systems	

4. Redox-sensitive polymers	
5. Bioadhesive system	Drug coated with bioadhesive polymer that selectively provides adhesion to colonic mucosa.
6. Coating of miroparticles	Drug is released through semi-permeable membrane
7. Osmotic controlled delivery	Osmotic pressure

CHEMICAL OR PRODRUG APPROACH:

A prodrug is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous enzymatic transformation *in vivo* to release the active drug as shown in **figure-5**. In this method the prodrugs are designed to undergo minimum absorption and hydrolysis in the upper GIT and undergo enzymatic hydrolysis in the colon, there by releasing the active drug moiety from the carrier. Different types of conjugates are used to prepare 5-ASA prodrugs, which are succeed in releasing the 5-ASA in colonic region. They are biodegradable poly (ether-ester) azo polymers[10] , azo-linked polymeric prodrugs[11] , acrylic type polymeric prodrugs and cyclo-dextrin prodrugs . Glucuronide prodrugs were developed for cortico-steriod to deliver the drug to the large intestine of colitic rats[12]. Azo-containing A urethane-based analogue containing an azo aromatic linkage in the backbone was synthesized by reacting touline-2, 6- di-isocyanate with a mixture of an aromatic azodiol[13].

Cyclodextrin prodrugs were prepared by conjugating 5-ASA on to the hydroxyl groups of α-, β-, γ-cyclodextris through an ester linkage and investigated the release in cecum and colon. After oral administration in rats the conjugate passed through stomach and small intestine without degradation or absorption and in the cecum and/or colon site-specific degradation of conjugate released 5-ASA. An azo prodrug of 5-ASA with histidine was synthesized for targeted drug delivery to the inflammated gut tissue in inflammatory bowel disease. The synthesized prodrug was found to be equally effective in mitigating the colitis in rats, as that of sulfasalazine without the ulcerogenicity of 5-ASA and adverse effective of Sulfasalazine .

In a recent study by Yunjin et al. (2006), explained the potential of 5- amino salicyliltaurine as a colon specific prodrug of 5ASA by in vivo evaluation to treat experimental colitis. The prodrug was prepared by conjugating 5ASA with taurine and tested in 2,4,6, trinitrobenzene sulfonicacid (TNBS) induced colitis rats. Taurine conjugation of 5-ASA greatly reduced absorption of 5-ASA from the intestine. Oral administration of the conjugate not only increased the colonic delivery efficiency of 5- ASA but also decreased the systemic absorption of free 5-ASA as compared to other conjugates prepared with glycine and aspartic acid. Taurine conjugate of 5-ASA is slightly more effective than sulfasalazine in alleviating the colonic inflammatory induced by TNBS. N-Nicotinoylglycyl-2-(5- fluorouracil-1-yl)-D,L-glycine was synthesized as a prodrug of 5-fluorouracil colon specific drug delivery.

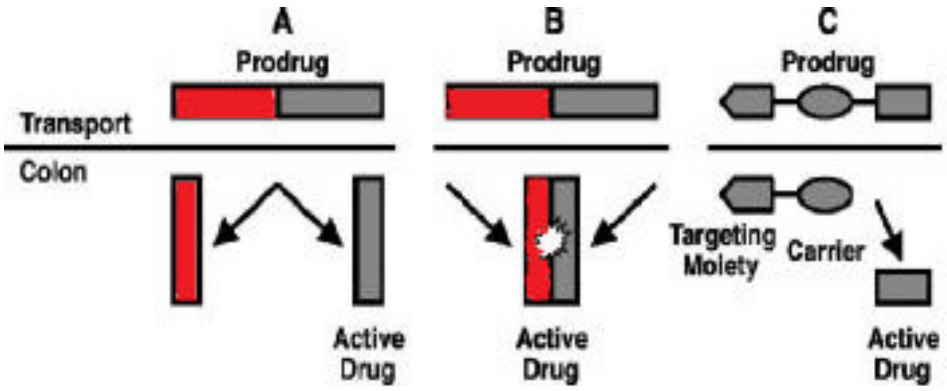


Figure 5: Prodrugs used for targeted drug delivery to colon

pH- DEPENDENT SYSTEM:

The basic principle in this method is the coating of the tablets/pellets etc with various pH sensitive polymers (given in **Table 3**), which will produce delayed release and also give protection from gastric fluids. The selected polymers to colon targeting should be able to withstand the pH of the stomach and small intestine. Methacrylic acid esters are the most

commonly used polymers for colon targeting because they are soluble at above pH 6. The ideal polymer should be able to withstand the lower pH of the stomach and of the proximal part of the small intestine but able to disintegrate at neutral or shortly alkaline pH of the terminal ileum and preferably at ileocecal junction. Eudragit L and Eudragit S are widely used in the colon targeting because Eudragit L is soluble at pH 6 or above and Eudragit S is soluble at pH 7 or above and the combination of these polymers give the desirable release rates.

A novel colon-specific drug delivery system was developed with methacrylate derivatives of 5-ASA using pH sensitive swelling and drug release properties. Composite film coated tablets of 5-ASA were prepared for colon specific delivery. In this method 5-ASA core tablets were prepared and coated with dispersion containing Eudragit RS and dessterrifed pectin, polygalacturonic acid, or its potassium and sodium salts. Negligible drug release occurred during first five hours where the coated tablets were in the stomach and small intestine. After that the release of 5-ASA from coated tablets occurred linearly as a function of time due to the action of pectinolytic enzymes [14].

A comparison study of the usual enteric-coated polymers viz. Eudragit, Cellulose acetate phthalate with Shellac and Ethyl cellulose as carriers for colon specific drug delivery was conducted to select a suitable carrier. In this study lactose based Indomethacin tablets were prepared and coated with one of the above coating polymers to a varying coating thickness. From the dissolution data at a coat concentration of 3% shellac provided the most appropriate polymer coat for colon-specific drug delivery. Variation in the shellac coat thickness can facilitate drug delivery to terminal ileum, distal or proximal colon [15].

EUDRACOLTM is a novel pH and time controlled multiple unit colon drug delivery systems in which the pellets coated with Eudragit RL /RS and Eudragit FS 30D. Caffeine is used as marker drug for pharmacokinetic studies using the multi particle principle and delayed release in the colon; reduction of dosing frequency may be achieved. Due to its specific coating structure, the Eudracol system offers a new dimension for colon drug targeting via the oral route [16]. 5-ASA pellets were coated with the enteric coating solution containing different ratios at Eudragit L-100 and Eudragit S-100 for colon drug delivery. The release of 5-ASA is depending on the thickness of the layer and the ratio of Eudragit copolymers[17]. pH-sensitive hydrogels were prepared for colonic delivery of therapeutic peptides, proteins. New pH-sensitive glycopolymers were developed by free radical polymerization of methacrylic acid

and 6-hexandiol diacrylate and 6- hexandiol propoxylate diacrylate[18].

Table 3: List of pH dependent polymers [19,20]

pH dependent polymers	**Threshold** pH
Polyvinyl acetate phthalate (PVAP) (Coateric®)	5.0
Cellulose acetate phthalate (CAP) (Aquateric®)	6.2
Cellulose acetate trimellitate (CAT)	5.5
Hydroxypropyl methylcellulose acetate succinate (HPMCAS) LF Grade MF Grade HF Grade	 ≥5.5 ≥6.0 ≥6.8
Hydroxypropyl methylcellulose phthalate (HPMCP) HP-50 HP-55 and HP-55S	 ≥5.0 ≥5.5
Shellac (MarCoat 125 & 125N)	7.0
Eudragit® FS 30D	≥7.0
Methacrylic acid copolymer, Type A (Eudragit®L-100 and Eudragit® L12, 5)	≥6.0
Methacrylic acid copolymer, Type B (Eudragit®S-100 and Eudragit® S12, 5)	≥7.0

Methacrylic acid copolymer, Type C (Eudragit® L100-55)	≥5.5
Methacrylic acid copolymer dispersion (Eudragit® L30D)	5.6

TIME-DEPENDENT SYSTEM

The basic principle involve in the system is the release of drug from dosage form should be after a predetermined lag time to deliver the drug at the right site of action at right time and in the right amount [21]. Colon targeting could be achieved by an incorporating a lag time into formulation equivalent to the mouth to colon transit time. A nominal lag time of five hours is usually considered sufficient to achieve colon targeting. In this method the solid dosage form coated with different sets of polymers (listed in **Table 4**) and the thickness of the outer layer determines the time required disperse in aqueous environment.

Colon drug delivery system of Diclofencac sodium was developed using a time dependent approach. In this, Diclofencac sodium tablets were coated with ethyl cellulose in ethanol solution cooling diethyl phthalate as a plasticizer and PEG 400 as channelling agent. The lag time of Diclofenac Sodium release was primarily controlled by thickness of ethyl cellulose coating layer. By increasing the thickness of an coating layer, longer the lag time of Diclofenac sodium release . Formulation of an fast release enteric coated tablets for colon drug delivery using two different approaches . In the first approach core tablets (celicoxib as a model drug) were prepared using different concentrations of super disintegrates like cross-linked PVP. In second approach concentrations tablets were prepared using potassium chloride, sodium chloride as osmogen. Then they are coated with Eudragit L-100: Eudragit S-100 in the ratio of 1:5 to achieve a desired thickness. The tablets with super disintegrates are fast released where the tablets with osmogen are sustain released. The coat weight determines the lag phase that required eliminating the release in stomach and small intestine.

Hydroxy Propyl Methyl Cellulose compression coated tablets of 5-fluorouracil were studied for colon drug delivery that based on time-dependent approach. In this, the core tablet was prepared by wet granulation method and then coated with 50% of HPMC/lactose coat powder by compression-coating method. Drug release characteristics were evaluated in distilled

water by using a Chinese pharmacopoeia rotatable basket method.

MICRO FLORA ACTIVATED SYSTEM

The principle involved in this method is degradation of polymers coated on the drug delivery system by microflora present in colon and there by release of drug load in colonic region because the bio environment inside the human GIT is characterized by presence of complex microflora, especially the colon is rich in microorganisms[22]. In this method drugs and dosage forms are coated with the biodegradable polymers **(Table 4)** i.e., the polymers degrade due to influence of colonic microorganisms. When the dosage form passes through the GIT, it remain intact in the stomach and small intestine where very little microbial degradable activity is present which is insufficient for cleavage of the polymer coating.5-ASA pellets was coated with amylose for colon drug delivery, in which amylose coating solution was prepared along with Ethocel , Eudragit RS/RL 30D and Aquacoat ECD 30[23]. Chitosan capsules was developed for colon specific delivery of insulin and its absorption was improved by addition of absorption enhancers (Sodium glycocholate, Sodium oleate) and protease inhibitors like bacitracin, aprotinin. Low swelling guar gum prepared by corsslinking with glutaraldehyde that is used as a colon-specific drug carrier. Chitosan succinate and chitosan phthalate were synthesized by reacting the chitosan separately with succinic anhydride and phthalic anhydride. These semi synthetic polymers produced stable matrices of Diclofencac sodium for colon specific delivery that had more resistance to acidic condition and improved drug release profile under basic conditions [24].

Organic acids like succinicacid, tartaricacid and citricacid were used as excipients in matrix granules to modify the drug release for colon-specific drug delivery. Amylose-Ethyl cellulose film coatings obtained from organic-based solvents were investigated as potential vehicles for colon drug delivery. In this method amyulose-butanol dispersion and ethyl cellulose in ethyl lactate/ethanol/propanol with dibutylsebacate as plasticizer were mixed in various proportions and coated on 5-ASA pellets to achieve desired thickness. The drug release regulating parameters are thickness of coating and ratio of amylose to ethyl cellulose. The release of drug is irrespective of the solvent used for coating. Formulation containing 1 part amylase and 1 part ethyl cellulose of coating thickness, 15% TWG, gives desired release profiles of 5-ASA for colon targeting[25].

Phosphated cross-linked guar gum was prepared for colon-specific drug delivery. Guar gum

cross-linked with increasing amounts of trisodiumtrimetaphosphate to reduce its swelling properties for use as a vehicle in oral delivery formulations, especially drugs aimed at localizing in the distal portions of the small bowel. Swelling of guar gum in artificial GI fluids was reduced from 100-120- fold to 10-35-fold depending on the amount of cross linker used [26].

Colon target drug delivery system for Mebendazole was developed using guar gum as a carrier. In this method Mebendazole matrix tablets containing various proportions of guar gum were prepared by wet granulation technique using starch paste as a binder. From the results 20% and 30% guar gum tablets were provided targeting of Mebendazole for local action in the colon. The α-cyclodextrin derivate of prednisolone-21-succinate showed anti-inflammatory activity with low adverse effects when compared to Prednisolone alone by intra colonical administration to rats with 2,4,6,trinitrobenzene sulfonicacid-induced colitis. The conjugate can alleviate the systemic adverse effect of prednisolone while maintaining the therapeutic activity of prednisolone [27].

A chitosan-dispersed system (CDS) was developed for colon- specific drug delivery, in which the capsule containing acetaminophen was coated with the suspension containing chitosan powder and Eudragit RS, formed a drug release-regulating layer around the capsule. Outer enteric coating layer prevent the dissolving of chitosan under acidic pH. The resultant enteric-coated CDS capsules reached the large intestine within one to three hours after oral administration and they were degraded at the colon in beagle dogs were studied about the lactulose as a carrier for colon-specific drug delivery by microbial degradation in colon. Enteric-coated pectin based matrix tablets were prepared for colonic delivery of Theophylline.

This approach takes advantage of the combination of pH-sensitive method and microbial-triggered system. In this method Theophylline-colon biodegradable pectin matrix tablets were prepared and coated with enteric coating solution (Eudragit S100 in acetone) to overcome the poor compatibility of pectin. Emdex, a hydrophilic directly compressible material was used to prepare tablets by direct compression. The new quaternized chitosan i.e. triethyl chitosan (TEC) is evaluated in pharmaceutical approaches and proved that there is a significant increase in absorption of poorly absorbed compounds in colon specific drug delivery

system[28].

Calcium pectinate beads was prepared for colon specific delivery of therapeutic peptides like bovine serum albumin (BSA) by extruding BSA-loaded pectin solution to an agitating calcium chloride solution and gelled spheres were formed instantaneously by an ionotropic gelation reaction.

The drug release was regulated by concentration of pectin, concentration of calcium chloride and total drug loading. The HPMA Copolymer (N-(2-hydroxy propyl) methacrylamide)-9 amino camptothein conjugate containing a spacer was synthesized and characterized for oral colon specific drug delivery. The drug delivery system has potential in the treatment of colon cancer [29]. Zinc pectinate beads formed the strongest network matrix in comparison with calcium pectinate and suggested the zinc pectinate beads as efficient carriers for specific drug delivery to colon [30].

Metronidazole tablets were prepared using various polysaccharides like guar gum, xanthan gum, pectin, carrageenan, β-cyclodextrin for colon specific drug delivery to treat ameobiasis[31]. 5-Fluorouracil compression coated tablets were prepared for colonic release of drug using xanthan gum, boswellia gum and HPMC as the coating materials [32]. CDDS of 5-fluorouracil was developed using pectin-ethyl cellulose as a film coat with FBC [33].

Table 4: Materials used in formulation of CDDS

Prodrug conjugates	**pH-Sensitive Polymers**	**Materials used In Time-Dependent System**	**Microbial degradable polymers**
Azo bond conjugates Amino acid (Polypeptide)	Eudragit L-100 Eudragit S-100	Hydroxy Propyl Methyl Cellulose Hydroxy Ethyl	Chitosan Pectins

conjugates	Eudragit L-30 D	Cellulose	Guar gum
Glycoside conjugates	Eudragit L-100-55	Ethyl Cellulose	Dextrans
Glucuronide conjugates and Sulphate conjugates	Eudragit F S 30 D	Microcrystalline Cellulose	Inulin
Polymeric conjugates	Poly Vinyl Acetate Phthalate	Hydroxy Propyl Methyl Cellulose Acetate Succinate	Lactulose
Cyclodextrin conjugates	Hydroxy Propyl Methyl Cellulose Phthalate 50	Lactose/Behinic acid	Amylose
Dextran conjugates	Hydroxy Propyl Methyl Cellulose Phthalate 55		Cyclodextrins
	Hydroxy Propyl Ethyl Cellulose Phthalate		Alginates
	Cellulose Acetate Phthalate		Locust bean gum
	Cellulose Acetate Trimellate		Chondroitin sulphate
			Boswellia gum

COMBINATION OF DIFFERENT APPROACHES OF CDDS :

An oral colonic drug delivery system of 5-ASA has developed using combination of pH dependent, time-based and enzyme degradable approaches. The pellets were coated with three functional layers i.e. the outer Eudragit L- 30D-55 layer for protection against GI fluids, the intermediate layer of ethyl cellulose to inhibit the drug release during passage through the small intestine and the inner layer of pectin for swelling and enzyme-degradation. In vitro release studies indicate that the coated pellets completely protected the drug release in 0.1M Hcl while the drug release was delayed for three to four hours in pH 6.8 phosphate buffer.

Pulsatile device was formulated to achieve a time or site-specific release of Theophylline based on chrono pharmaceutical consideration. The basic design consists of an insoluble hard gelation capsule body filled with Eudragit microcapsules of Theophylline and sealed with a hydrogel plug and finally the enteric device was enteric coated. In this approach, pH sensitive and time dependent delivery systems were combined. In this the thickness of enteric coat is a measure of protection from stomach and intestine pH. Different hydrogel polymers were used as plugs to maintain a suitable lag period.The hydrophilic polymer content is a measure of delayed release of theophylline from microcapsules [34].

Pectin based CDDS of 5-fluorouracil was developed using calcium pectinate gel. Calcium pectinate gel beads were prepared by ionotropic gelation method followed by enteric coating with Eudragit S-100 and evaluated using USP paddle type dissolution apparatus in different simulated mediums [35].

A new microbial-triggered colon targeted osmotic pump (MTCT-OP) was developed for CDDS based on chitosan for a model drug, Budesonide. The combination of osmotic technology and microbial-triggered mechanism had a high potential to deliver to drug load in colonic region. In this method the core tablet of Budesonide was prepared with chitosan, which is used to produce osmotic pressure, and to form the *insitu* delivery pores for colon-specific drug release. Cellulose acetate in acetone along with chitosan (as pore forming agent) was coated on tablet as a semipermeable membrane and finally coated with Eudragit L-100-55 in ethanol as an enteric coating layer that could prevent cellulose acetate membrane from forming pore or rupture before reaching colon region. Budesonide release from developed system was inversely proportional to the osmotic pressure to the release medium[36].

HYDROGEL BASED CDDS:

Amylated pectin hydrogel beads prepared for colon specific delivery of Indomethacin and Sulfamethoxazole[37]. Glutaraldehyde cross-linked dextran capsules were prepared for colon targeting. Along with magnesium chloride and PEG 400 in water the capsule caps and bodies were prepared on nylon molding pins. Then the dextran capsules were filled with model drug (Hydrocortisone) and drug release was studied. The drug release pattern was suitable for colon specific delivery [38]. The hydrogels formed by cross-linked polyvinyl alcohol were suitable for colon specific drug delivery systems. In this method polyvinyl alcohol of

different molecular weights was corss-linked with succinyl, adipoyl, or sebacoyl chloride to obtain hydrogel-forming polymers. The hydrophilic drugs like Diclofencac sodium, propranolol hydrochloride and vitamin B6 hydrochloride were used as model drugs[39]. Methacrylated inulin hydrogels designed for colon targeting the proteins like Bovine serum albumin or Lysozyme. Organic redox-initiated polymerization technique was used to fabricate pH responsive hydrogels for colon specific delivery [40].

Glutaraldehyde corss-linked guar gum hydrogel discs were prepared as vehicles for colon specific drug delivery of ibuprofen. Percent of drug release increased with glutaraldehyde concentration. Cross-linking decreased the swelling of guar gum. The fabricated hydrogels discs may prove to be beneficial as colon-specific drug delivery vehicles for poorly water-soluble drugs like ibuprofen [41].

Novel complex hydrogel beads were prepared using pectin and zein for colon-specific drug delivery. Pectin/Zein complex hydrogel beads showed the capability to protect incorporated drugs from premature release into stomach and small intestine. The inclusion of a small portion of zein (a protein from corn) in to the pectin efficiently suppressed the swelling behavior of pectin, thus stabilizing the structural property of the pectin networks. Likewise the pectin networks protected the bound zein from protease digestion. These properties made pectin/zein complex beads a promising system for colon specific drug delivery [42]. Cross-linked HPMC hydrogels were synthesized and used to develop 5-ASA colon drug delivery system [43].

NOVEL DRUG DELIVERY SYSTEMS FOR CDDS:

Now a days the basic CDDS approaches are applied to formulate novel drug delivery systems like Multi particulate systems, Microspheres, Liposomes, Microencapsulated particles etc.

Multi particulate systems

Multiparticulates (pellets, non-peariles etc.) are used as drug carriers in pH-sensitive, time-dependent and microbially control systems for colon targeting. Multi particulate systems have several advantages in comparison to the conventional single unit for controlled release

technology, such asmore predictable gastric emptying and fewer localized adverse effect than those of single unit tablets or capsules.

A multi particulate dosage form was prepared to deliver active molecules to colonic region, which combines pH dependent and controlled drug release properties. This system was constituted by drug loaded cellulose acetate butyrate (CAB). Microspheres loaded by an enteric polymer (Eudragit S). Here the enteric coating layer prevents the drug release below pH 7. After that CAB microspheres efficiently. controlled the release of Budesonide, which is depended on the polymer concentration in the preparation . Azo polymer coated pellets were used for colon-specific drug delivery to enhance the absorption of insulin and Eel Calcitonin. A multi particulate chitosan dispersed system (CDS) was prepared for colon drug delivery and it was composed of the drug reservoir and the drug release-regulating layer, which was composed of water insoluble polymer and chitosan powder. The drug reservoir was prepared by drug containing multiparticulates like Non-peariles in the study. In this study the multi particulate CDS was adopted not only for colon specific drug delivery but also for sustained drug delivery.

A multi particulate system combining pH sensitive property and specific biodegradability was prepared for colon targeted delivery of Metronidazole. The multi particulate system was prepared by coating cross-linked chitosan microspheres explorting Eudragit L-100 and S-100 as pH sensitive polymers. The *in-vitro* drug release studies shows that no release of drug at acidic pH and higher drug release was found in presence of rat caecal contents indicating susceptibility of chitosan matrix to colonic enzymes released from rat caecal contents. High-Amylose cornstarch and Pectin blend micro-particles of Diclofencac sodium for colon-targeted delivery were prepared by spray drying technique. The blending of high-amylose cornstarch with pectin improved the encapsulation efficiency and decreased the drug dissolution in the gastric condition from pectin based micro-particles. The drug released in colonic region by the action of pectinase from micro-particles [44]. Investigated the effect of sodium glycocholate as an absorption promoter on orally administrated insulin absorption utilizing a colon-targeted delivery system. A novel insulin colon-targeted delivery system (Insulin- CODES) contains insulin, lactulose as a trigger for colon-specific release, citricacid as a solubilizer of insulin, meglumine as a pH adjusting agent and sodium glycocholate as an absorption promoter.

Microspheres of anti-cancer drugs:

Cross-linked guar gum microspheres containing Methotrexate were prepared and characterized for local release of drug in the colon for efficient treatment of colorectal cancer. In this method glutaraldehyde was used as a cross-linking agent and guar gum microspheres were prepared by emulsification method. From the results of *in vitro* and *in vivo* studies the Methotrexate loaded cross linked guar gum microspheres delivered most of the drug load (79%) to the colon, where as plain drug suspensions could deliver only 23% of three total dose to the target tissue [45]. Colon specific microspheres of 5-fluorouracil were prepared and evaluated for the treatment of colon cancer. In this method core microspheres of alginate were prepared by modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The results showed that this method had great potential in delivery of 5-fluorouracil to the colon region[46].

Advantages of microspheres:

1. Provide selective passive targeting to tumour tissues.
2. Flexibility to couple with site-specific ligands to achieve active targeting.
3. Increased efficacy and therapeutic index.
4. Increased stability via encapsulation.
5. Reduction in toxicity of the encapsulated agent.
6. Improved pharmacokinetic effects.

EVALUATION OF CDDS:

The drug release in the colonic region from different CDDS is evaluated by different methods of *in vitro* and *in vivo* release studies, which show the success rate of different designs of colon drug delivery systems. Depending upon the method of preparation different evaluation methods are proposed. A successful colon specific drug delivery system is one of that remains intact in the physiological environment of stomach and small intestine, but releases the drug in the colon.

***In-vitro* Evaluation:**

Different in vitro methods are used to evaluate the colonic drug delivery systems. In *in-vitro* studies the ability of the coats/carriers to remain intact in the physiological environment of the stomach & small intestine is assessed by drug release studies in 0.1N Hcl for two hours (mean gastric emptying time) and in pH 7.4 phosphate buffer for three hours (mean small intestine transit time) using USP dissolution apparatus. In case of micro flora activated system dosage form, the release rate of drug is tested in vitro by incubating in a buffer medium in the presence of either enzymes (e.g. pectinase, dextranase) or rat/guinea pig rabbit caecal contents. The amount of drug released at different time intervals during the incubation is estimated to find out the degradation of the carrier under study [47].

***In-vivo* Evaluation:**

The successful development of the CDDS is ultimately determined by its ability to achieve release in colonic region thus exerts the intended therapeutic effect. When the system design is concerned & prototype formulation with acceptable *in-vitro* characteristics is obtained*, in vivo* studies are usually conducted to evaluate the site specificity of drug release and to obtain relevant pharmacokinetic information of the delivery system. Although animal models have obvious advantages in assessing colon specific drug delivery systems, human subjects are increasingly utilized for evaluation of this type of delivery systems. The preferable animals to evaluate CDDS are rats, guinea pigs and dogs.[48] γ-scintgraphic studies were conducted in human volunteers with technetium-99m-DTPA as tracers in sodium chloride core tablets compression coated with guar gum showed that the gum coat protect the drug (tracer) from being released in the stomach and small intestine. On entering the ascending colon, the tablets commenced to release the tracer indicating the breakdown of gum coat by the enzymatic action of colonic bacteria.[49] Technetium-99m-DTPA was used as a tracer for γ- scintigraphy evaluation of colon specific guar gum directly compressed matrix tablets in human volunteers.[50] The scintigraphic evaluation conducted for capsule type colon specific drug delivery system in human healthy volunteers.[51] In a study by Krishnaiah et al. (2001),[54] showed the effect of Metronidazole and Tinidazole (antimicrobial agents) on the release of Albendazole from guar gum based colon specific matrix tablets. The active antimicrobial agents (7 days) treatment of rat caecal content decreased the release of Albendazole due to decreased levels of anaerobic bacteria present in rat.

Table 5: Marketed colon specific drug delivery systems

Drug	**Trade Name**	**Coating Polymers**
Mesalazine	Claversa	Eudragit L 100
	Asacolitin	Eudragit S
	Mesazal	Eudragit L 100
	Asacol	Eudragit S
Budesonide	Entrocort	Eudragit L100-55
	Budenofalk	Eudragit S
	Targit	Coated Starch Capsule
Sulfasalazine	Azulfidine	Cellulose acetate phthalate
	Colo-Pleon	Eudragit L100-55

REVIEW OF LITERATURE

- **HN Shivakumar et al., (**2006) [52] prepared the system comprising of Eudragit S-100 coated pellets was designed for chronotherapeutic delivery of Diltiazem hydrochloride. The drug loaded core pellets were produced by aqueous extrusion spheronization technique using microcrystalline cellulose as a spheronizing aid and PVP K 30 as a binder. Different coat weights of Eudragit S-100 were applied to the drug loaded pellets in an automatic coating machine to produce the pH sensitive coated pellets. Pellets performed following pH progression method showed that the drug release from the coated pellets depended on the coat weights applied and pH of the dissolution media.
- **Nagpal et al., (**2006) [53] synthesized a prodrug of 5-ASA with histidine for targeted drug delivery to the inflammated gut tissue in inflammatory bowel disease. The synthesized prodrug was found to be equally effective in mitigating the colitis in rats, as that of Sulfasalazine without the ulcerogenecity of 5-ASA and adverse effects of Sulfasalazine.
- **Prabal Kumar et al.,** (2007) [54] prepared Megaloporous controlled release tablets of Diclofenac sodium (DS) were prepared with two kinds of granules. One of them is the restraining-phase matrix granule (RMG) and it controls the release rate of the drug. The other one is the soluble housing-phase matrix granule (HMG) and controls liquid penetration into the system. Carnauba wax and Eudragit L 100 polymers were used to constitute the restraining and housing matrix phases, respectively. *In vitro* drug release study was carried out in simulated gastric fluid (pH 1.2) for the first 2 h and in phosphate buffer (pH 7.2) for the next 10 h following USP 25 paddle method. The fabricated megaloporous matrix tablets released only 3 to 5% of DS in pH 1.2 depending on the proportion of carnauba wax used in the RMG.
- **Parasuram Rajam Radhika et al.,** (2008) [55] formulated colon specific aceclofenac microspheres as Aceclofenac delayed release microspheres employing cellulose acetate phthalate as enteric polymer. The effect of various other modern enteric polymers HPMCP, Eudragit S 100 and L 100 on the release of aceclofenac from the CAP microspheres has been evaluated. The studies revealed that Eudagit polymers exhibited negative effect on the release of CAP microspheres.

- **Naikwade Sonali R et al.,** (2008) [56] formulated matrix tablets of Tinidazole employing swellable polymers (HPMC K4M & K15M) and Eudragit (S-100 & L-100) which were then enteric coated to provide localised action in colon. Bioavailability study showed that greater portion of Tinidazole was released in Large intestine and drug level in plasma was above 4 µg/mL in blood for 24 hrs

- **Amal H. El-Kamela et al.,** (2008) [57] prepared oral colon targeted delivery systems for treatment of inflammatory bowel disease: Synthesis, *in vitro* and *in vivo* assessment and investigated the potential of prodrugs of some non-steroidal anti-inflammatory drugs (NSAIDS) as colon targeted delivery systems for treatment of inflammatory bowel diseases. Naproxen, Sulindac and Flurbiprofen (Fbp) were used.

- **Mohanad Naji Sahib et al.,** (2009) [58] formulated Prednisolone as an oral modified release tablet for colonic targeting. Many trials were performed to prepare a satisfactory formula using the wet granulation method with various additives and coatings. The formula containing 1% Eudragit RS PM was the best with regard to 100% release of drug in comparison with other concentrations and other retardant types. Eudragit S 100 provided the best release of drug in pH 7.4 phosphate buffer.

- **Patel S.N et al.,** (2009) [59] developed colon specific drug delivery of Prednisolone sustained release matrix tablets for ulcerative colitis using HPMC K-4M and HPMC K-100M as a semi synthetic polymer. Prednisolone based tablets were coated using methacrylic acid copolymers Eudragit® S100 by spraying organic system. The matrix tablets of Prednisolone are subjected to an *in vitro* drug release study using simulated colonic fluid of pH 7.2 as the dissolution medium. The results also demonstrated that a Eudragit® S100 can be successfully used for organic system to coat tablets for colon targeted delivery of drug.

- **Sateesh Kumar Vemula et al.,** (2010) [60] describes on different approaches to design and evaluation of colon specific drug delivery systems. Different approaches are designed based on prodrug formulation, pH sensitivity, time-dependency (lag time), microbial degradation and osmotic pressure etc to formulate the different dosage forms like tablets, capsules, multiparticulates, microspheres, liposomes for colon targeting. The efficiency of drug delivery system is evaluated using different *in vitro* and *in vivo* release studies. This review updated that the research on different approaches for formulation and evaluation of colon-specific drug delivery systems (CDDS). In 1942, Svartz discovered that Sulfasalazine; the sulphanilamide prodrug of 5-aminosalicylic acid (5-ASA) is effective in the treatment of rheumatoid arthritis and anti-inflammatory disease. The exact mode by which the drug target itself to the colon was elucidated much later in 1970 i.e., colon specific azoreductase splits Sulfasalazine causing the release of the active moiety 5-amino salicylic acid. After the several other azo-bonds containing compounds designed to locally release 5-aminosalicylic acid were synthesized Bensalazine, Balsalazide, Olsalazine.

- **R. Thiruganesh et al.,** (2010) [61] formulated Colon specific release systems of Aceclofenac by loading Aceclofenac into pectin pellets followed by coating the loaded pellets with pH dependent polymeric coating solution containing Eudragit L 100 and S 100 (1:4). Pellets containing four proportions of pectin were prepared. Eudragit coated pectin pellets prevented release of the Aceclofenac in the physiological environment of stomach and small intestine depending on the proportion of pectin used in the formulation.

- **MS Khan et al.,** (2010) [62] developed and evaluated multiparticulates of alginate and chitosan hydrogel beads exploiting pH sensitive property for colon-targeted delivery of theophylline. Alginate and chitosan beads were prepared by ionotropic gelation method followed by enteric coating with Eudragit S100. The studies showed that formulated alginate and chitosan beads can be used effectively for the delivery of drug to colon and a coat weight of 20% weight gain was sufficient to impart an excellent gastro resistant property to the beads for effective release of drug at higher pH values.

- **M. N. Patro et al.,** (2010) [63] developed pH - sensitive polymers Eudragit S 100 and L 100 to prepare microspheres of 5-Fluorouracil by a simple oil /water emulsification process. In further attempts mixtures with Eudragit S100 and L100 were prepared to

prolong drug release. Eudragit S100, pure or in mixture, was found to retain drug release at pH 4.5 lower than 41% within 6 hrs. At pH 7.4, nearly immediate release (within 30 min) was observed for pure S100, while mixtures enabled to prolong the release slightly.

- **Gaurav Tiwari et al.,** (2010) [64] prepared Primary and novel approaches for colon targeted drug delivery which includes prodrugs, pH and time dependent systems, microbially triggered drug delivery system and their limitations. Newly developed CDDS, which includes pressure controlled colonic delivery capsules (PCDCS), CODESTM and osmotic controlled drug delivery are unique in terms of achieving *in vivo* site specificity and feasibility of manufacturing process. This review also focuses on evaluations of CDDS in general.

- **S. Jose et al.,** (2010) [65] developed chitosan microspheres of Ondansetron by emulsion cross linking method for colon targeted delivery intended to treat irritable bowel syndrome combining pH dependent solubility by Eudragit S100 and microbial degradability of chitosan polymers. The optimized formulation was subjected to microencapsulation using Eudragit S100 by solvent evaporation technique. Formulation containing 1:10 core/coat ratio released lesser amount of drug in upper GIT.

- **Kulkarni p keshavaro et al.,** (2011) [66] formulated colon specific Indomethacin microspheres as Indomethacin delayed release microspheres. Using pH sensitive polymers Eudragit S 100 and L 100. *In vitro* drug release study was carried out in pH 6.8 for 12 hours the percentage of drug release was found to be 95.58%. Concluded that the Indomethacin microspheres can be successfully designed to develop sustained drug delivery.

- **Jitender mor et al.,** (2011) [67] developed for colon targeted delivery systems, The colon route can be employed both local and systemic delivery of drugs. Treatment can be made effective if the drugs can be targeted directly into the colon. He has mentioned that the average ph of colon at various regions are ascending colon 6.4 and transverse colon 6.0-7.4 and descending colon 6.0-7.4 pH and transit time of dosage

form in GIT (hrs) in stomach <1(fasting),>3(fed) and in small intestine 3-4 ,in large intestine (20-30).

AIM & OBJECTIVE

Aim:

The aim of this research is to design and evaluate novel Colon Specific Release Systems of Aceclofenac using pH dependent polymer .When Aceclofenac is administered as the conventional formulation, it causes gastro intestinal complications including irritation, ulcer, bleeding and perforation. Site-specific delivery of drugs to the site of action has the potential to reduce side effects and to increase pharmacological response. Incorporation of pH dependent polymer in the core tablet, minimizes all these complications which makes it suitable candidate for administration by oral route.

Objective:

- To develop pH dependent aceclofenac tablets with a view of minimizing the drug release in the physiological environment of stomach and small intestine and to ensure maximum drug release in the physiological environment of colon with an improved patient compliance. Incorporating Eudragit S 100 as a pH dependent polymer in the core tablet.
- Aceclofenac is used for treating ulcerative colitis , rheumatoid arthritis & osteoarthritis, which had apparent circadian rhythms and peak symptoms in the early morning.
- When Aceclofenac is administered orally as a conventional formulation, it is difficult to achieve the desired clinical effect, because it elicits patient's non-compliance of administration in the early morning to co-ordinate the rhythm of rheumatoid arthritis & osteoarthritis, due to rapid absorption of conventional formulation.
- Aceclofenac is also used in the treatment of inflammatory bowel disease. Aceclofenac, a non-steroidal anti-inflammatory drug exhibits better tolerance than Aspirin, Indomethacin and Naproxen.
- To study release profile of colon specific dosage form for 12 hrs.
- To perform the stability studies for the prepared formulation.

PLAN OF WORK

The present research work is planned according to the following steps:

1. Preformulation studies
2. Drug – Excipient Compatibility Studies by DSC and FT-IR.
3. Preparation of granules of Aceclofenac by Wet Granulation using Eudragit S-100 dissolved in isopropyl alcohol as binder and by increasing Eudragit S-100 in linear fashion, followed by lubrication.
4. Pre-compression testing of the prepared granules for bulk density, tapped density, compressibility index, angle of repose and Hausner's ratio followed by compression of the tablets.
5. Evaluation of the prepared tablets includes
   a) Physical parameters like hardness, friability, weight variation and drug content .
   b) In-vitro dissolution studies
6. Optimization of the tablet by determining the trial that shows satisfactory release profile in in-vitro release tests.
7. Stability studies as per the ICH guidelines for a period of 30 days.

DRUG PROFILE

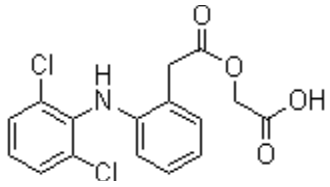
ACECLOFENAC :

Aceclofenac is an orally administered phenylacetic acid derivative with effects on a variety of inflammatory mediators. It is from the class of non-steroidal anti-inflammatory drug (NSAID), related to Diclofenac.

Chemical IUPAC Name: 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid

Chemical Formula: $C_{16}H_{13}Cl_{12}NO_4$

Chemical Structure:



Molecular Weight: 354.18

State: Solid

Melting Point: 149 - 153^{0}c

Solubility: Practically insoluble in water, freely soluble in acetone, soluble in alcohol.

Hydrophobicity: 3.03

Drug category: Analgesic agent, Anti-inflammatory agent

Indication: Acute pain in, Osteoarthritis, Rheumatoid arthritis, ulcerative colitis, Low back pain, Dental Pain, Fracture and painful Pharyngitis & Tonsillitis.

Pharmacology: Aceclofenac is a new phenylacetic acid derivative provided with marked anti-inflammatory, anti-arthritic, analgesic and antipyretic activities in animal experimental models. While maintaining its potency, Aceclofenac demonstrates better gastric tolerance and consequently offers greater potential security than other highly active agents such as Indomethacin and Diclofenac.

PHARMACOKINETICS:

Absorption:

After oral administration, Aceclofenac is rapidly absorbed and the bioavailability is almost 100%. Peak plasma concentrations are reached approximately 1.25 to 3 hours following ingestion. T max is delayed with food intake whereas the degree of absorption is not influenced.

Serum Protein Binding: 99.70%

Biotransformation: Aceclofenac is probably metabolized via CYP2C9 to the main metabolite 4-hydroxyaceclofenac.

Site of Metabolism: Liver

Tmax: 1.25 to 3 hours

Cmax: Chronic heavy alcohol abusers may be at risk of liver toxicity from excessive aceclofenac use. Renal impairment patients with mild renal function should be monitored regularly since the use of NSAIDs may result in deterioration of renal function.

Half Life: 4 to 4.3 hours

Dose: 100 mg once a day

Food Interactions: Tmax is delayed with food intake where as degree of absorption is not influenced.

Bioavailability: 100%

Excretion: Approximately two-third of the administered dose is excreted via the urine, mainly as conjugated hydroxy metabolites. Only 1% of an oral single dose is excreted unchanged. A slower rate of elimination of Aceclofenac has been detected in patients with decreased liver function after a single dose of Aceclofenac. In a multiple dose study using 100 mg once daily, there was no difference in the pharmacokinetic parameters between subjects with mild to moderate liver cirrhosis and normal subjects. In patients with mild to moderate renal impairment, no clinically significant differences in the pharmacokinetics were observed after a single dose.

Pharmacodynamics:

Aceclofenac is a novel NSAID known to exhibit multifactor mechanism of action. Aceclofenac was developed in order to provide a highly effective pain relieving therapy with a reduced side effect profile.

1. Aceclofenac directly blocks PGE 2 secretion at the site of inflammation by inhibiting IL-Beta & TNF in the inflammatory cells (Intracellular Action). Aceclofenac has been demonstrated to inhibit cyclooxygenase (COX) activity and to suppress the PGE-2 production by inflammatory cells, which are likely to be a primary source of PGE-2. Inflammatory cells release IL-1 and TNF, which produce PGE 2 by induction of COX-2. Aceclofenac and 4'-hydroxyaceclofenac penetrate the inflammatory cells like polymorphonuclears, monocytes and rheumatoid synovial cells and get hydrolyzed to the active metabolites Diclofenac and 4'-hydroxydiclofenac which inhibit IL-1 and TNF released by the inflammatory cells and therefore suppress production of PGE 2 at the site of inflammation.
2. Aceclofenac stimulates the synthesis of the extracellular matrix of the Human Articular Cartilages. Aceclofenac blocks degeneration and stimulates synthesis of extracellular matrix of cartilages by inhibiting the action of different cytokines. Aceclofenac and the metabolites inhibit IL-6 production by human chondrocytes. This leads to inhibition of increase of inflammatory cells in synovial tissue, inhibition of IL-1 amplification, inhibition of increased MMP synthesis and thus ensuring proteoglycan production. Aceclofenac also inhibits IL-1 and TNF production by human chondrocytes, inflammatory cells and synovial cells and therefore blocks suppression of GAG and collagen synthesis and stimulates growth factor mediated synthesis of GAG and collagen. 4'-hydroxyaceclofenac, a metabolite of aceclofenac inhibits pro MMP1 and pro MMP3 produced by synovial cells (Rheumatoid Synovial Cells) in serum and in synovial fluid and thus inhibits progressive joint destruction by MMPs.
3. Aceclofenac inhibits Neutrophil adhesion & accumulation at the inflammatory site in the early phase and thus blocks the pro-inflammatory actions of Neutrophils.

EXCIPIENTS PROFILE

1. **Microcrystalline Cellulose (Diluent)**
2. **Eudragit S100 (pH dependent Polymer)**
3. **Magnesium Stearate (Lubricant)**
4. **Talc (Glidant)**

MICROCRYSTALLINE CELLULOSE [68]

Synonyms: Avicel, Cellulose gel, Crystalline cellulose, E460, Emocel; Fibrocel; Vivacel.

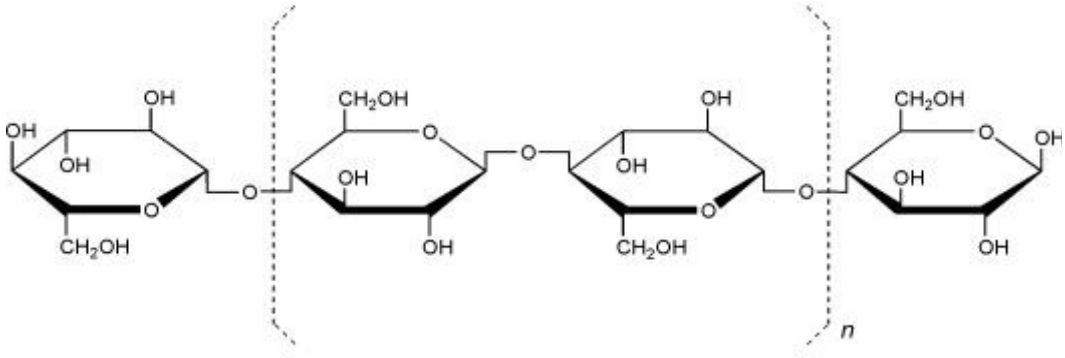
Non-proprietary Name:

BP :- Microcrystalline cellulose.

Chemical Name: Cellulose

Emperical Formula: ($C_6H_{10}O_5$)

Structure:



Molecular Weight: ≈36000

Functional Category:

- Absorbent, Suspending agent
- Tablet and Capsule- Diluent

Description: White-colored, tasteless crystalline powder composed of porous particles.

Solubility: Slightly soluble in 5% w/v sodium hydroxide solution, practically insoluble in water, dilute acids and most organic solvents.

Application in pharmaceutical formulation or technology:

Microcrystalline cellulose is widely used in pharmaceuticals primarily as a diluents in oral tablets and capsule formulations where it is used in both wet granulation and direct compression processes. Microcrystalline cellulose also has some lubricant and disintegrant properties that make it useful in tableting and also used in cosmetics and food products.

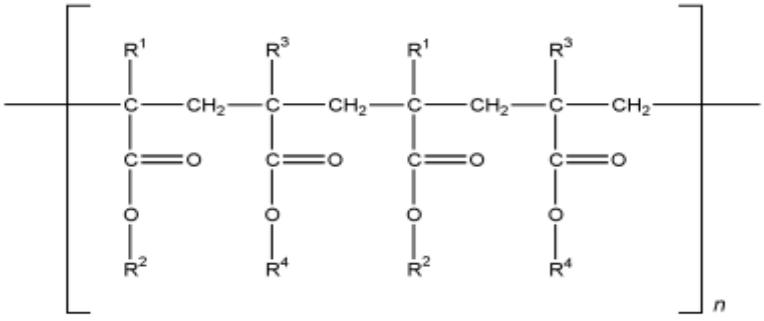
Stability: It is a stable, though it is hygroscopic material.

Storage conditions: The bulk material should be stored in a well-closed container in a cool, dry place.

Incompatibilities: Incompatible with strong oxidizing agents.

EUDRAGIT [69,70]

Structure :



$R_1 = CH_3$; H

$R_2 = CH_3$

R_3 = COOH; Eudragit L & S

$R_4 = CH_2CH_2N^+(CH_3)Cl$; Eudragit RL & RS

Advantages for coating:

- ❖ pH-dependent drug release
- ❖ Protection of actives sensitive to gastric fluid
- ❖ Protection of gastric mucosa from aggressive actives
- ❖ Increase in drug effectiveness
- ❖ Good storage stability
- ❖ GI and colon targeting

Table 6: Threshold pH of Eudragit polymers:

Polymer	Threshold pH
Eudragit® L100	6.0
Eudragit® S100	7.0
Eudragit® L30D	5.6
Eudragit® FS 30D	6.8
Eudragit® L100-55	5.5

MAGNESIUM STEARATE [71]

Synonyms : HyQual, Magnesium octadecanoate, Stearic acid magnesium salt.

Chemical Name : Octadecanoic acid magnesium Salt

Emperical Formula : $C_{36}H_{70}MgO_4$

Molecular Weight : 591.27

Functional category : Lubricant

Applications : It is used as a lubricant in capsule and tablet manufacture at concentrations between 0.25-5.0%.

Description : It is a fine, white, precipitated or milled, impalpable powder of low bulk density, having a faint characteristic odour and taste. The powder is greasy to touch and readily adheres to the skin.

Solubility : Practically insoluble in ethanol, ethanol (95%), ether and water, slightly soluble in benzene and warm ethanol (95%).

Stability : Magnesium stearate is stable.

Storage conditions : It should be stored in a well-closed container in a cool, dry place.

Incompatibilities : Incompatible with strong acids, alkalis, iron salts and with strong oxidizing materials.

Safety : It is generally regarded as being non-toxic following oral administration. However, oral consumption of large quantities may result in some laxative effect or mucosal irritation.

TALC [71]

Synonyms : *Magsil Osmanthus, Magsil Star, Purtalc, Steatite.*

Emperical formula : $Mg(Si_2O_5)_4(OH)_4$

Functional category :

- Glidant
- In Tablet and capsule lubricant
- Anti-cacking agent

Applications : It is used as a lubricant in solid dosage forms (1-10%) and in topical preparations as dusting powder (90-99%).

Description : It is a very fine, white to greyish-white colored, odourless, impalpable, unctuous powder. It adheres to the skin, is soft to touch, and free from grittiness.

Solubility : Practically insoluble in dilute acids and alkalies, organic solvents and water.

Stability : Talc is a stable material.

Storage conditions : It should be stored in a well-closed container in a cool, dry place.

Incompatibilities : Incompatible with quaternary ammonium compounds.

Safety : Following oral ingestion talc is not absorbed systemically and may thus be regarded as an essentially nontoxic material.

MATERIALS

Table No 7: Materials used:

S.NO	MATERIALS USED	
1	Aceclofenac	Aurobindo pharma Ltd, Hyderabad
2	Microcrystalline Cellulose	Accent Microcel Industries
3	Eudragit S100	Corel Pharma Chem
4	Croscarmellose sodium	FMC BioPolymer
5	Magnesium stearate	Peter greven
6	Purified Talc	Luzonac pharma
7	Iso Propyl Alcohol	Ranchem
8	Hydrochloric acid	SD fine chemical limited
9	Potassium dihydrogen orthophosphate	SD fine chemical limited

INSTRUMENTS USED:

Table no 8: Instruments used :

S.No	Instruments Used	Maker
1	Digital balance	Sartorius Cp 323S
2	Drier	Retsch, TG - 100
3	Kalaweka blender	Cadmach machinery co. pvt. Ltd, HD-410 AC, Ahmadabad
4	RMG granulator	Gansons high speed mixer granulator, HSMG2
5	Differential scanning calorimetry	Perkin Elmer Thermal Analyzer
6	Tablet compressor	Cadmach machinery co. pvt. Ltd, Ahmadabad
7	Hardness tester	Varian, VK 200
8	Digimatic vernier callipers	Mitutoyo corporation, CD – 6''C
9	Friabilator	Electro lab EF-2, friabilator(USP), Mumbai
10	Tap density apparatus, USP	Electrolab, ETD – 1020, Mumbai
11	FT-IR	Schimadzo ST EQ-025
12	Digital tablet dissolution apparatus, USP	Electrolab, TDT – O8L Mumbai
13	Mechanical stirrer	Remi motors ltd, RQT – 124A, Mumbai
14	Moisture Analyzer	Sartorius, MA – 100
15	UV/Visible spectrophotometer	Shimadzu, A11454783115
16	pH meter	Electrolab, Mumbai

EXPERIMENTAL PROCEDURE:

DRUG – EXCIPIENT COMPATIBILITY STUDIES:

Drug instability is the loss of drug through chemical and physical degradation resulting in a reduction potency. To investigate the possible interaction between Aceclofenac and the excipients used in the formulations and to check the physical appearance of the product. The accurately weighed amount of drug and the excipient are kept into vial for 30days at 40°±2/75±5% RH 1 month**.** To check the physical appearance of the drug-excipient whether it is compatible.

DIFFERENTIAL SCANNING CALORIMETRY STUDIES (DSC):

In order to investigate the possible interaction between Aceclofenac, Eudragit S100, HPMCP, differential scanning calorimetry (DSC) analysis was carried out on pure substances and their physical mixtures (PM) in equimolar ratios using the Perkin Elmer Thermal Analyzer instrument equipped with a computerized data section. Samples (3 to 4 mg) were placed in an aluminium pan and heated at a rate of 10^0c/min with indium in the reference pan in an atmosphere of nitrogen at a rate of 50.ml/min to a temperature of 200^0c.

DRUG – EXCIPIENT INTERACTION STUDIES (FT-IR):

Infrared spectroscopy is one of the analytical technique for the determination of presence of various functional groups involved in making up the molecule. It provides very well accountable spectral data regarding any change in the functional group characteristics of a drug molecule occurring in the process of formulation. IR spectra of Aceclofenac and its formulations were obtained by Potassium bromide pellet method using FT-IR Schimadzo ST EQ-025 Spectrophotometer in order to find out drug-carrier interaction occurring during the formulation process.

EVALUATION TESTS:

PREFORMULATION EVALUATION TESTS

Determination of Bulk density and Tapped density:

An accurately weighed quantity of the powder (W), was poured into the graduated cylinder and the volume (V_0) was measured. Then the graduated cylinder was closed with lid, set into the density determination apparatus. The density apparatus was set for 100 taps and after that, the volume (V_f) was measured and operation was continued till the two consecutive readings were equal. The bulk density and tapped density were calculated using the following formula:

$$\text{Bulk density} = W / V_0$$

$$\text{Tapped density} = W / V_f$$

Where, W = Weight of the powder V_0 = Initial volume V_f = Final volume

Compressibility Index or Carr's Index (CI):

It indicates the ease with which a material can be induced to flow. The Compressibility Index (Carr's Index) is a measure of the propensity of a powder to be compressed. It is determined from the bulk and tapped densities. the less compressible a material, the more flowable it is. It is a measure of the relative importance of inter-particulate interactions. In a free-flowing powder, such interactions are generally less significant and the bulk, tapped densities will be closer in value. For poor flowing materials, there are frequently greater inter-particle interactions and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index which is calculated using the following formula:

$$\mathbf{CI = \frac{(D_t - D_b)}{D_t} \times 100}$$

Where, D_t = Tapped density of the powder.

D_b = Bulk density of the powder.

Hausner's Ratio:

Hausner's ratio was measured by the ratio of tapped density to bulk density.

Hausner's ratio = Tapped density / Bulk density

Table 9: Carr's Index and Hausner's Ratio values

Compressibility Index	Flow character	Hausner Ratio
< 10	Excellent	1.00 – 1.11
11 – 15	Good	1.12 – 1.18
16 – 20	Fair	1.19 – 1.25
21 – 25	Passable	1.26 – 1.34
26 – 31	Poor	1.35 – 1.45
32 – 37	Very poor	1.46 – 1.59
> 38	Very, Very poor	> 1.60

Angle of Repose (θ):

The frictional forces in a loose powder can be measured by angle of repose, θ. This is the maximum angle possible between the surface of a pile of powder and the horizontal plane. The powder mixture was allowed to flow through the funnel fixed to a stand at definite height. The angle of repose was then calculated by measuring the height and radius of the heap of the powder formed.

The fixed funnel method was employed to measure the angle of repose. A funnel was secured with its tip at a given height (h), above a graph paper that is placed on a flat horizontal surface. The blend was carefully poured through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius (r) of the base of the conical pile was measured. The angle of repose (θ) was calculated using the following formula:

$$\theta = \tan^{-1}(h/r)$$

Where, θ = angle of repose, h = height of the pile in cms, r = radius of the pile.

COMPRESSION OF TABLET:

After Preformulation studies, the blend was subjected to compression using Cadmach 16-station machine and 7.5 mm diameter round standard concave punch. The hardness was maintained between 5-6 kg/cm^2 as the tablets were intended for controlled release and the tablets were subjected to the following post formulation evaluation tests.

POST FORMULATION EVALUATION TEST OF THE TABLETS

Hardness:

Hardness of the tablet is defined as the force applied across the diameter of the tablet in the order to break the tablet. The resistance of the tablet to chipping, abrasion or breakage under condition of storage transformation and handling before usage depends on its hardness. For each formulation, the hardness of 6 tablets was determined using a Varian ,VK 200 .hardness tester. It is expressed in **Kg / cm^2.**

Friability (F):

It is a measure of mechanical strength of tablets. The friability of the tablet was determined using Electro lab EF-2, friabilator (USP). It is expressed in percentage (**%**).It should be preferably between o.5 to 1.0%. 10 tablets were initially weighed ($W_{initial}$) and transferred into the friabilator. The friabilator was operated at 25 rpm for four mins. The tablets were weighed again (W_{final}). The percentage friability was then calculated by:

$$F = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100$$

Weight Variation:

Twenty tablets were selected randomly and weighed individually to check the weight variation. IP limit for weight variation in case of tablets weighing up to 80 mg is ± 10%. %Deviation = (Individual weight – Average weight / Average weight) x 100

Table 10: Pharmacopoeial specifications for tablet weight variation

Average weight of Tablets (mg) (I.P)	Average weight of Tablets (mg) (U.S.P)	Maximum percentage difference allowed
Less than 80	Less than 130	10
80 – 250	130 – 324	7.5
More than 250	More than 324	5

Thickness:

Tablet thickness is an important characteristic in reproducing appearance. Twenty tablets were taken and their thickness was measured by Digimatic vernier callipers. It is expressed in the form of mm.

Content Uniformity:

- Powder the tablets and weigh the powder equivalent to 100 mg of Aceclofenac taken in100 ml volumetric flask.
- Make up to 100 ml with methanol.
- Pipette out 2 ml into 100 ml flask and make up to 100 ml with methanol (20 ppm).

Construction of standard calibration curve of Aceclofenac:

Preparation of 7.4 pH Phosphate buffer:

7.4 pH phosphate buffer was prepared by taking 6.8 g of Potassium dihydrogen phosphate was accurately weighed and transferred into 1 litre volumetric flask and the volume was made up to 1 litre with distilled water and the pH is adjusted to 7.4 using sodium hydroxide solution.

Preparation of Standard Stock Solution:

22.4 mg of Aceclofenac standard was accurately weighed and transferred into 100 ml volumetric flask and the volume was made up to 100 ml with methanol.

Preparation of working standard solution:

- 5 ml of stock solution was pipetted into 100 ml volumetric flask and the volume was made up to 100 ml with 7.4 pH Phosphate buffer.
- 7.5 ml of stock solution was pipetted into 100 ml volumetric flask and the volume was made up to 100 ml with 7.4 pH Phosphate buffer.
- 5 ml of stock solution was pipetted into 50 ml volumetric flask and the volume was made up to 50 ml with 7.4 pH Phosphate buffer.
- 6.25 ml of stock solution was pipetted into 100 ml volumetric flask and the volume was made up to 50 ml with 7.4 pH Phosphate buffer.
- 7.5 ml of stock solution was pipetted into 100 ml volumetric flask and the volume was made up to 50 ml with 7.4 pH Phosphate buffer.

Preparation of Aceclofenac Tablets (F1-F5):

The formulation consists of active moiety Aceclofenac, microcrystalline cellulose as directly compressible diluents, croscarmellose sodium as disintegrating agent, talc as glidant and magnesium stearate as lubricant. The drug, microcrystalline cellulose ,Eudragit S-100 were passed through 40# size mesh separately prior to the preparation of the dosage form. Accurately weighed amount of drug, microcrystalline cellulose were mixed thoroughly for 10 min to ensure uniform mixing in geometrical ratio in Kalaweka blender . The obtained blend was subjected to granulation using Eudragit S100 dissolved in sufficient quantity of Isopropyl alcohol as binder. The obtained granules were subjected to drying in a dryer at 60^0c for 1 hour. The dried granules are then passed through 20# size mesh and then mixed with magnesium stearate, talc passed through 60# size mesh. The obtained blend was then subjected to preformulation studies.

Table 11: Formulation of Aceclofenac tablets (F1 –F5):

S.NO	Ingredients	F1(mg/tab)	F2(mg/tab)	F3(mg/tab)	F4(mg/tab)	F5(mg/tab)
1	Aceclofenac	100	100	100	100	100
2	MCC	17.60	22.60	27.60	32.60	37.60
3	Eudragit S100	40.00	35.00	30.00	25.00	20.00
4	IPA	Q.S	Q.S	Q.S	Q.S	Q.S
5	Talc	1.60	1.60	1.60	1.60	1.60
6	Magnesium stearate	0.80	0.80	0.80	0.80	0.80
7	Core Tablet weight	160.00	160.00	160.00	160.00	160.00

Determination of λ_{max} of Aceclofenac in 0.1 N Hcl:

Preparation of Simulated gastric fluid (0.1 N Hcl): 8.5 ml of concentrated hydrochloric acid was added in 1 litre volumetric flask and then volume was made up to 1 litre with distilled water and the pH was adjusted to 1.2 with concentrated hydrochloric acid.

Preparation of Standard Stock Solution:

22.4 mg of Aceclofenac standard was accurately weighed and transferred into 100 ml volumetric flask and the volume was made up to 100 ml with methanol .

Preparation of working standard solution:

- 5 ml of stock solution was pipetted into 100 ml volumetric flask and the volume was made up to 100 ml with 0.1 N Hcl.

Scanning range: 200 – 300 nm

Determination of λ_{Max} of Aceclofenac in 6.8 pH phosphate buffer:

Preparation of (6.8 pH phosphate buffer):

6.8 g of Potassium dihydrogen phosphate was accurately weighed and transferred into 1 litre volumetric flask and the volume was made up to 1 litre with distilled water and the pH is adjusted to 6.8 using sodium hydroxide solution.

Preparation of Standard Stock Solution:

22.4 mg of Aceclofenac standard was accurately weighed and transferred into 100 ml volumetric flask and the volume was made up to 100 ml with methanol.

Preparation of standard solution :

5 ml of stock solution was pipetted into 100 ml volumetric flask and the volume was made up to 100 ml with 6.8 pH phosphate buffer.

Scanning range : 200 – 300 nm

Determination of λ_{max} of Aceclofenac in 7.4 pH phosphate buffer:

Preparation of (7.4 pH phosphate buffer):

6.8 g of Potassium dihydrogen phosphate was accurately weighed and transferred into 1 litre volumetric flask and the volume was made up to 1 litre with distilled water and the pH is adjusted to 7.4 using sodium hydroxide solution.

Preparation of Standard Stock Solution:

22.4 mg of Aceclofenac standard was accurately weighed and transferred into 100 ml volumetric flask and the volume was made up to 100 ml with methanol.

Preparation of working standard solution:

5 ml of stock solution was pipetted into 100 ml volumetric flask and the volume was made up to 100 ml with 7.4 pH phosphate buffer.

Scanning range: 200 – 300 nm

Dissolution profile of the tablets:

- Dissolution of Aceclofenac tablets was studied using USP type 2 (paddle) dissolution test apparatus Digital tablet dissolution apparatus, Electrolab, TDT – O8L employing paddle as a stirrer in 900 ml of 0.1 N Hcl (1.2 pH) for 2 hours.
- It is carried out in 6.8 pH phosphate buffer for 3 hours and 7.4 pH phosphate buffer for 12 hours.
- The stirrer was adjusted to rotate at 75 rpm and a temperature of 37±0.5^{0}c was maintained throughout the experiment.

5 ml of samples were withdrawn at various time intervals and analyzed for Aceclofenac by measuring the absorbance at 276 nm. The volume withdrawn at various intervals was immediately replaced with fresh quantity of dissolution medium.

Stability studies:

- The optimized formulation were subjected to stability studies at 40°C ±2 / 75%±5 RH for 1month .
- The product was evaluated for the physical characteristics, drug content and *In vitro* drug release profile over a period of 1month

RESULTS

Table 12: DRUG – EXCIPIENT COMPATIBILITY STUDIES:

S.No	Ingredients	mg/tab	Drug excipient ratio	Observation	
				Initial	40±2°c/75±5%RH After 1- Month
1	Aceclofenac	100	-	White granular powder	No characteristic change
2	MCC	16	1 : 0.16	White granular powder	No characteristic change
3	Eudragit S100	40	1 : 0.4	White granular powder	No characteristic change
4	Talc	1.6	1: 0.016	White granular powder	No characteristic change
5	Magnesium stearate	0.8	1: 0.008	White granular powder	No characteristic change

DIFFERENTIAL SCANNING CALORIMETRY (DSC) STUDIES:

Figure 6: DSC of Pure drug Aceclofenac:

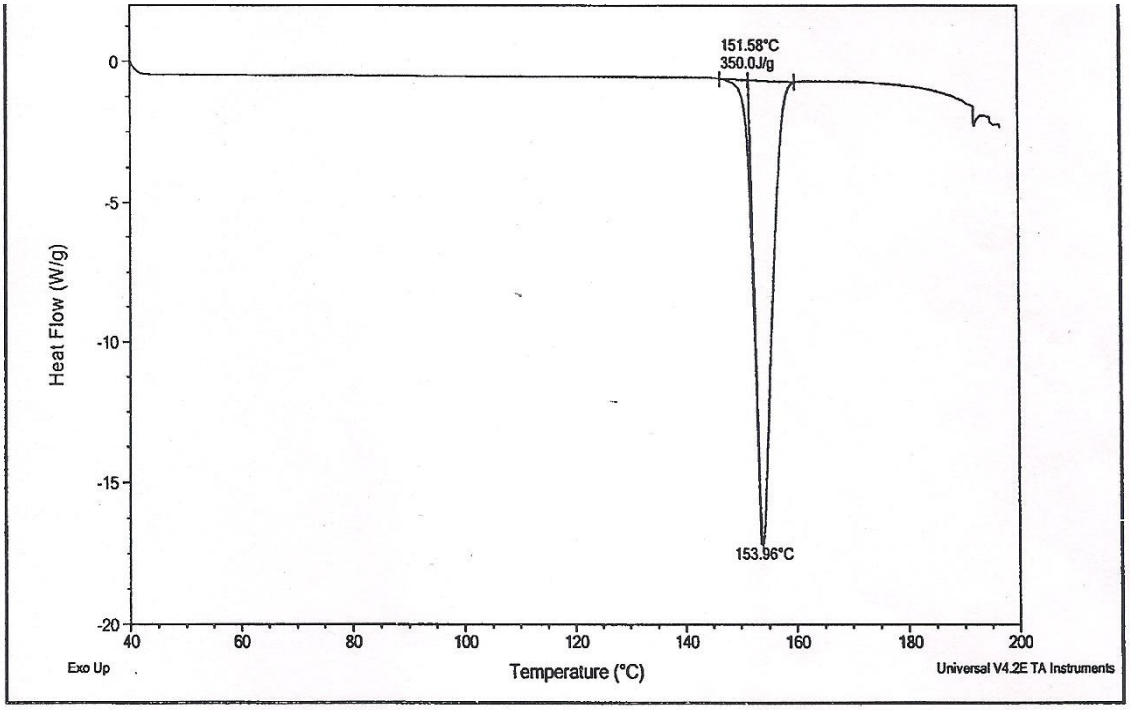


Figure 7: DSC of polymer Eudragit S100

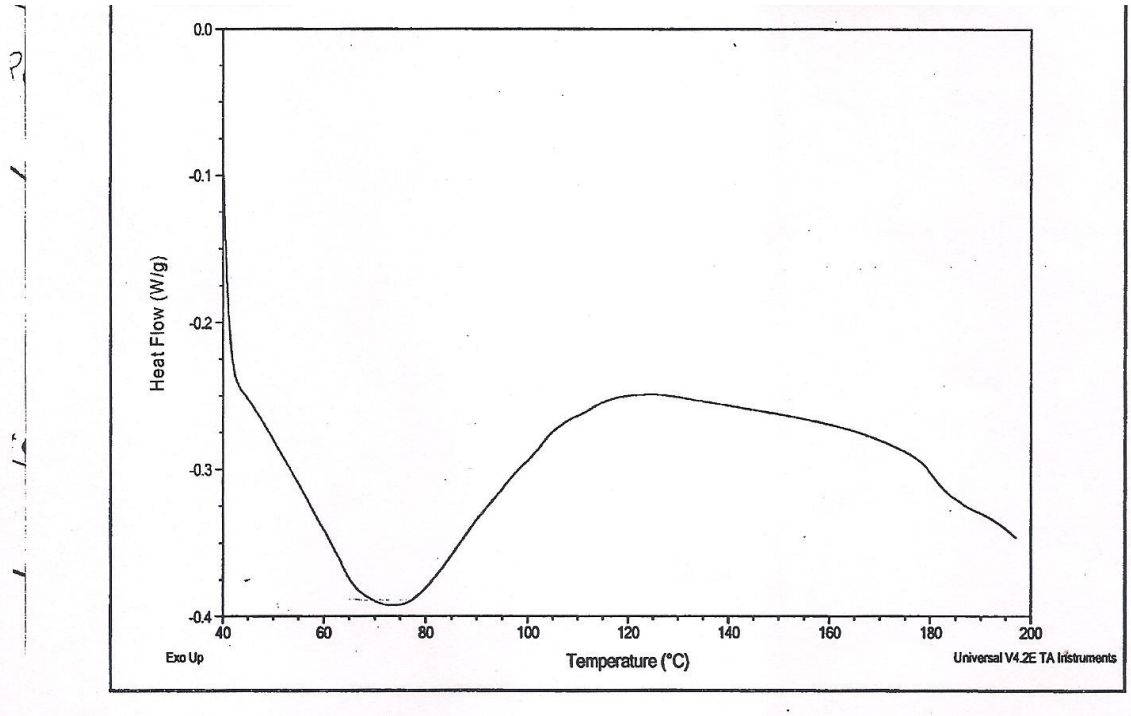


Figure 8: DSC of Aceclofenac and Eudragit S100

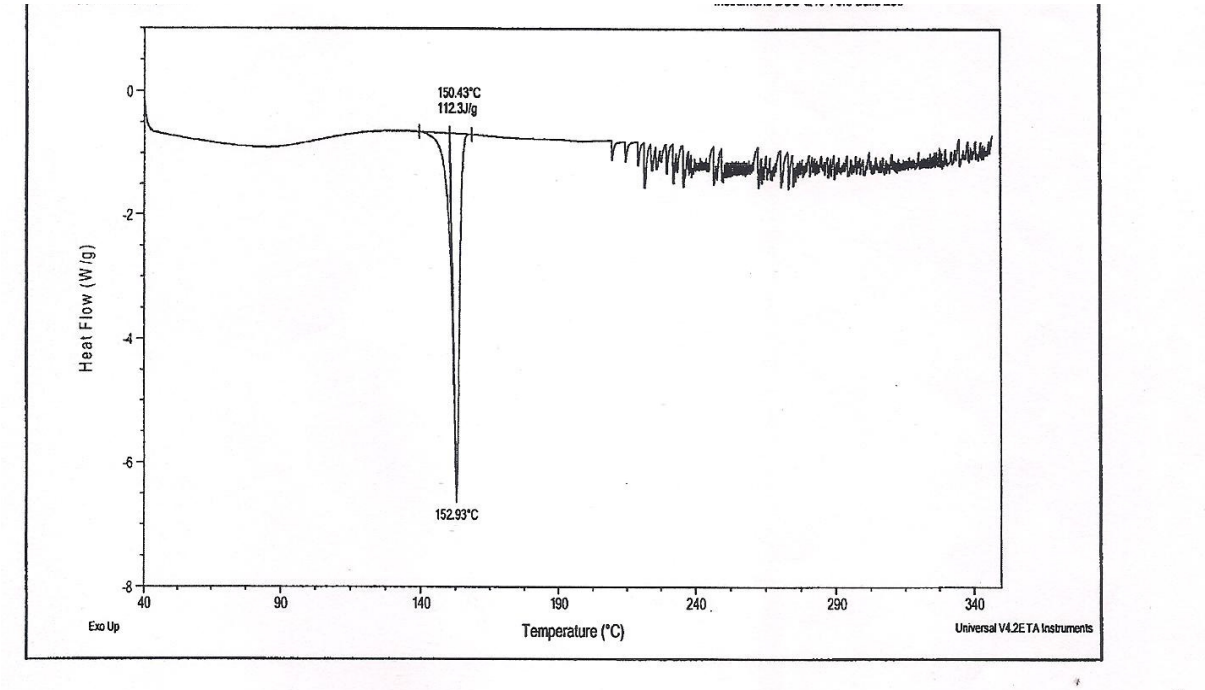


Table 13: Data obtained from DSC Curves:

S.No	Name of the substance	Peak Integration
1	Aceclofenac	Onset – 151.96°C.,Maximum – 153.96°C., Stop – 159.94°C
2	Eudragit S-100	No results to report
3	Aceclofenac + Eudragit S-100	Onset – 150.43°C., Maximum – 152.93°C., Stop – 158.31°C

DRUG – EXCIPIENT INTERACTION STUDIES (FT-IR):

Figure 9: FT-IR of Pure Aceclofenac

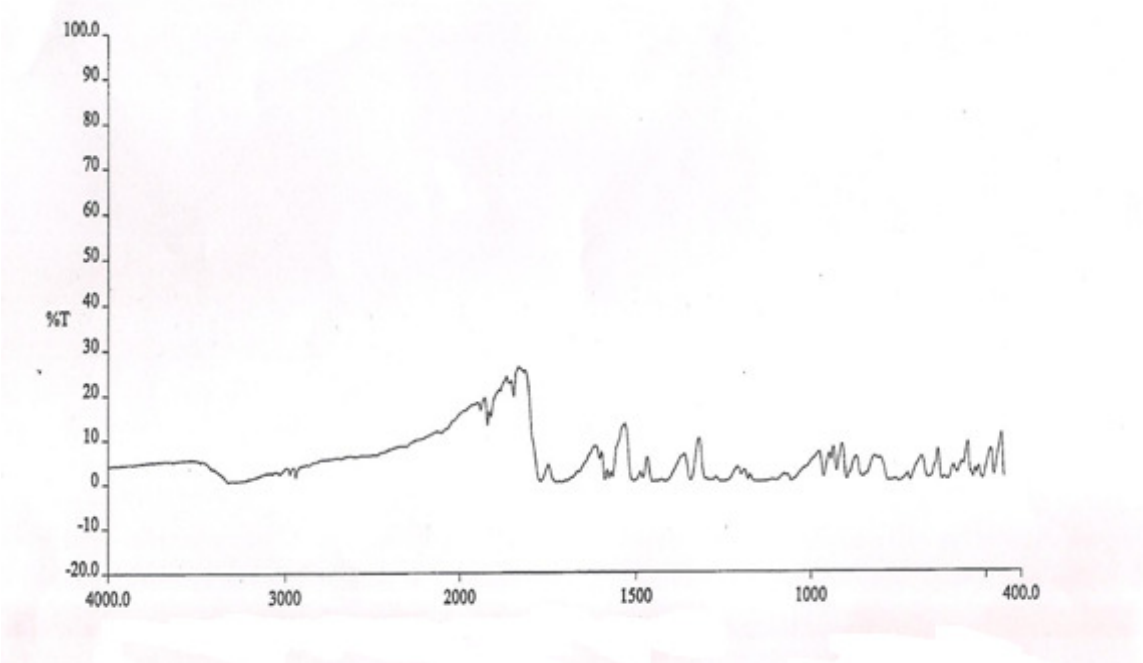


Figure 10: FT-IR of Aceclofenac & final (F-4):

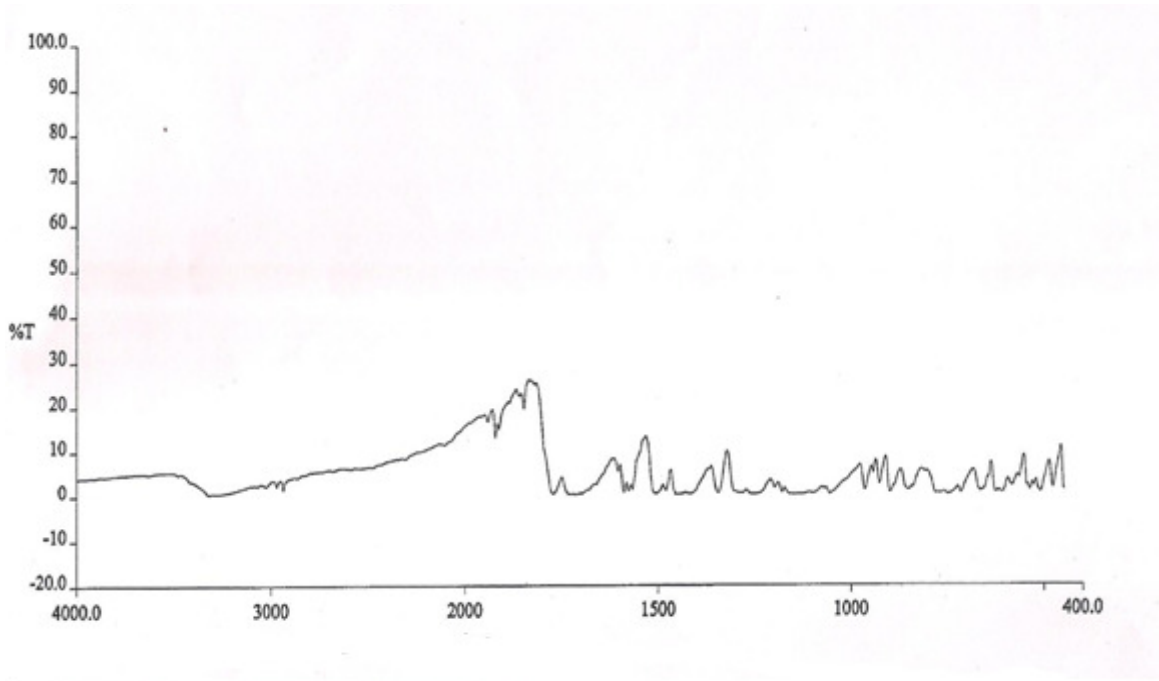


Figure 11: Comparision of FT-IR of Pure Aceclofenac and its final (F-4):

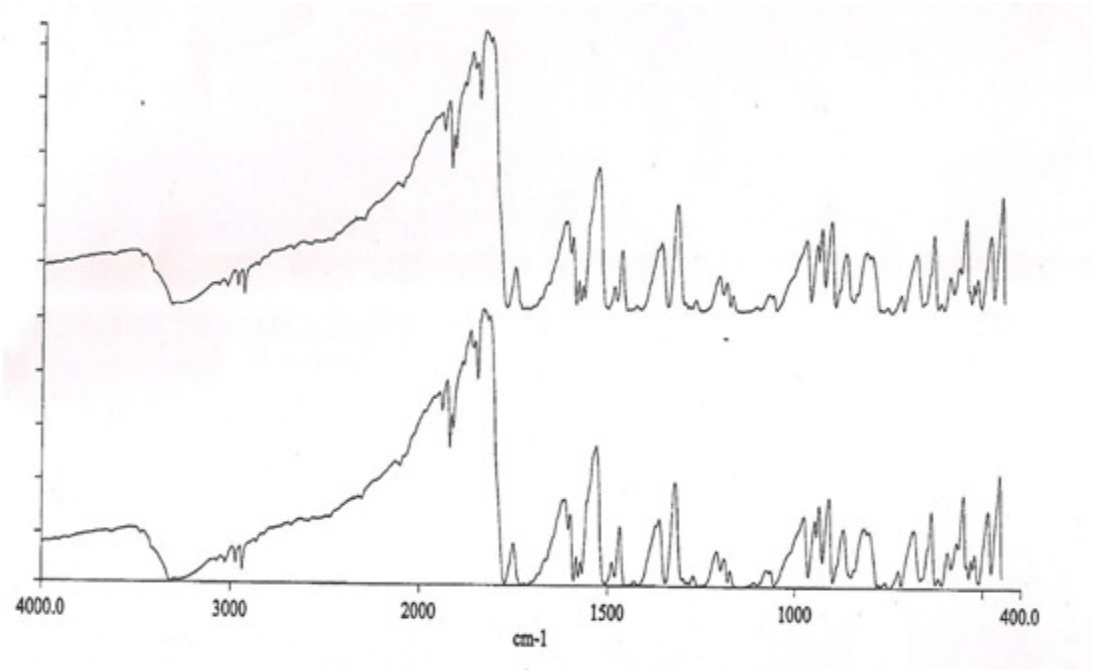


Table 14: Evaluation of Pre-formulation parameters of Aceclofenac tablet F1-F5:

Formulation Code	Angle of Repose ± S.D	Bulk Density	Tapped Density	% Carr's Index	Hausner's ratio
F1	30.32 ± 0.33	0.332	0.412	19.417	1.24
F2	32.07 ± 0.66	0.344	0.436	21.100	1.26
F3	30.12 ± 0.33	0.365	0.469	22.174	1.26
F4	29.33±0.33	0.323	0.398	18.844	1.23
F5	27.32 ± 0.14	0.325	0.405	19.753	1.24

Table 15: Evaluation of Post-Compression parameters of Aceclofenac tablets:

Formulation Code	Hardness (kg/cm^2)	Average weight of 20 tablets (mg)	Friability (%)	Thickness (mm)	Drug Content ± S.D (%)
F1	5.5	163.3 ± 0.33	0.38	3.2	100.6 ± 0.74
F2	5.0	160.5 ± 0.35	0.45	3.2	99.9 ± 0.70
F3	5.0	162.0 ± 0.25	0.58	3.2	97.2 ± 0.28
F4	5.5	160.4 ± 0.56	0.64	3.2	98.6 ± 0.61
F5	6.0	161.5 ± 0.64	0.54	3.2	99.3 ± 0.58

*Average of three determinations.

Table 16: Calibration and absorbance values of Aceclofenac in 7.4 pH phosphate buffer at 276 nm:

S.No	Concentration (mcg)	Absorbance (nm)
1	0	0
2	0.0112	0.277
3	0.0168	0.419
4	0.0224	0.553
5	0.0280	0.693
6	0.0336	0.830

Figure 12: Standard Calibration curve of Aceclofenac in 7.4 pH Phosphate buffer:

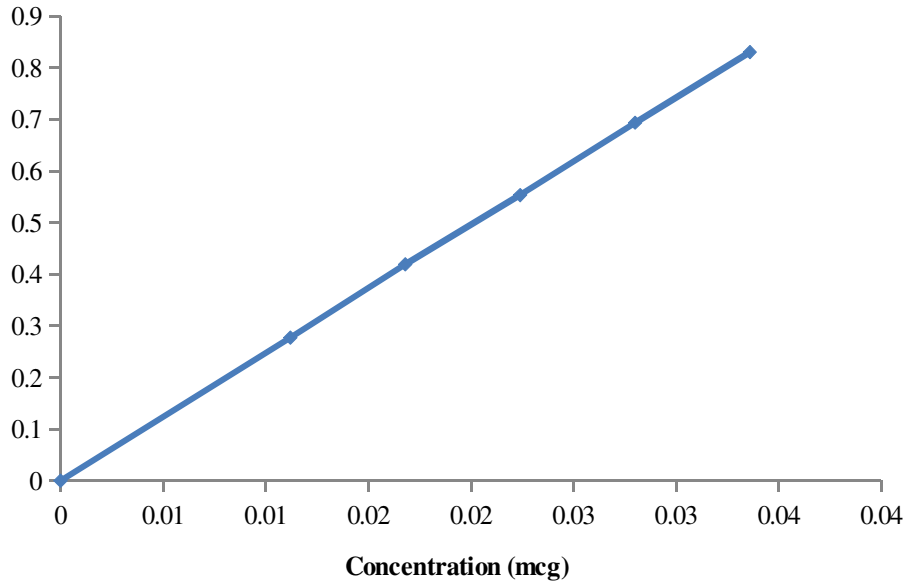


Table 17: Determination of λ_{max} of Aceclofenac in 0.1 N Hcl:

S.No	Wavelength(nm)	Absorbance
1	250.0	0.0260

2	255.0	0.0290
3	260.0	0.0393
4	265.0	0.0534
5	270.0	0.0664
6	275.0	0.0695
7	280.0	0.0569
8	285.0	0.0268
9	290.0	0.0085

Figure 13: Determination of λ_{max} of Aceclofenac in 0.1 N Hcl:

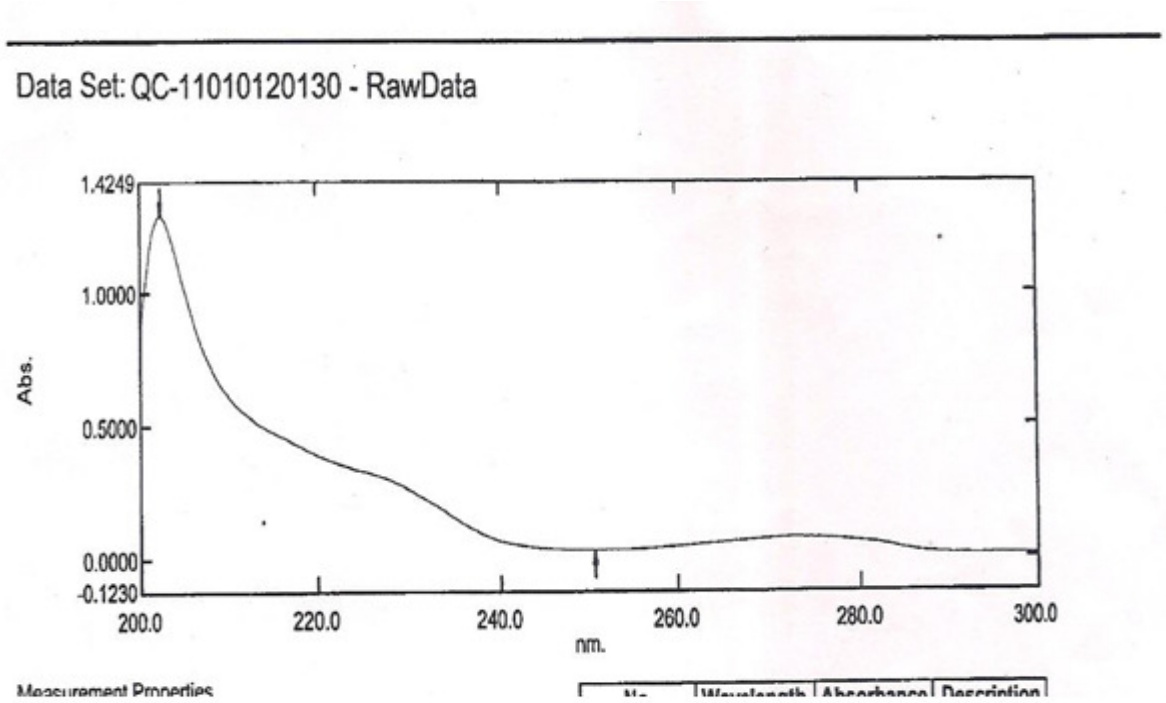


Table 18: Determination of λ_{max} of Aceclofenac in 6.8 pH phosphate buffer:

S.No	Wavelength(nm)	Absorbance
1	250.0	0.0162
2	255.0	0.0193
3	260.0	0.0253
4	265.0	0.0434
5	270.0	0.0557
6	275.0	0.0595

7	280.0	0.0462
8	285.0	0.0154

Figure 14: Determination of λ_{max} of Aceclofenac in 6.8 pH phosphate buffer:

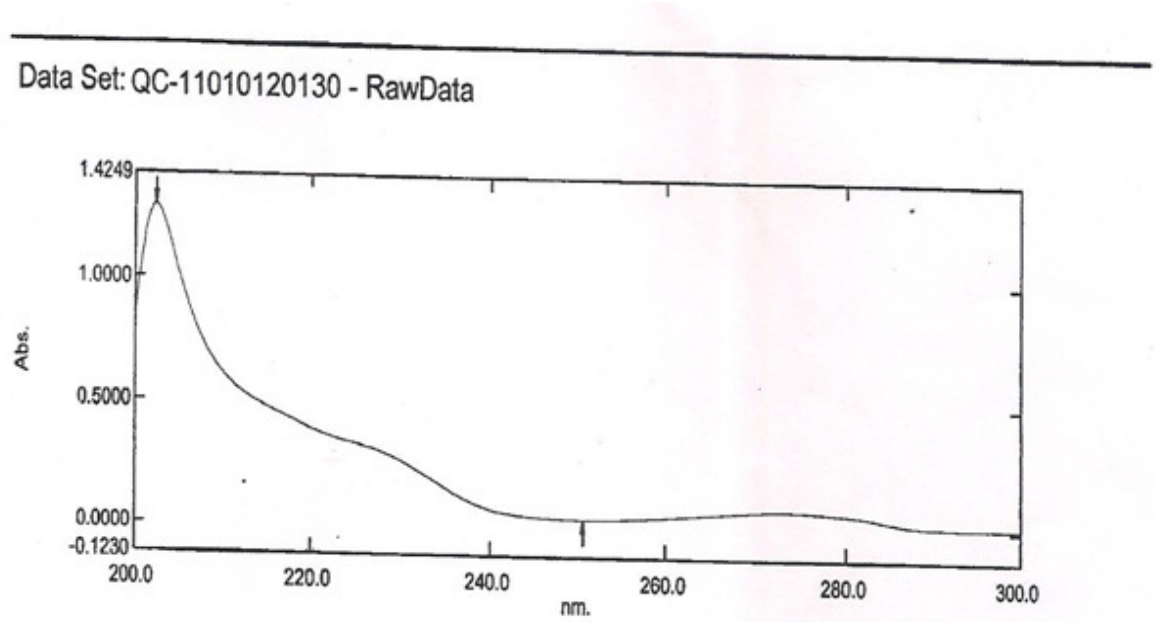
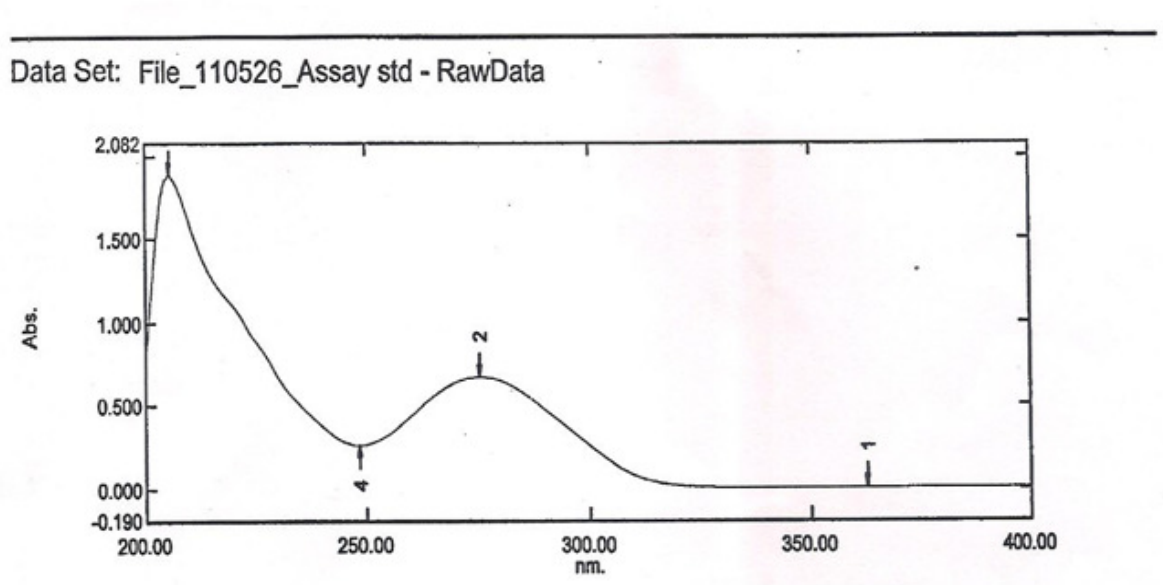


Table 19: Determination of λ_{max} of Aceclofenac in 7.4 pH phosphate buffer:

S.No	Wavelength (nm)	Absorbance
1	271.0	0.645
2	272.0	0.659
3	273.0	0.666
4	274.0	0.669
5	275.0	0.671
6	276.0	0.671
7	277.0	0.669
8	278.0	0.664
9	279.0	0.654

10	280.0	0.648

Figure 15: Determination of λ_{max} of Aceclofenac in 7.4 pH phosphate buffer:



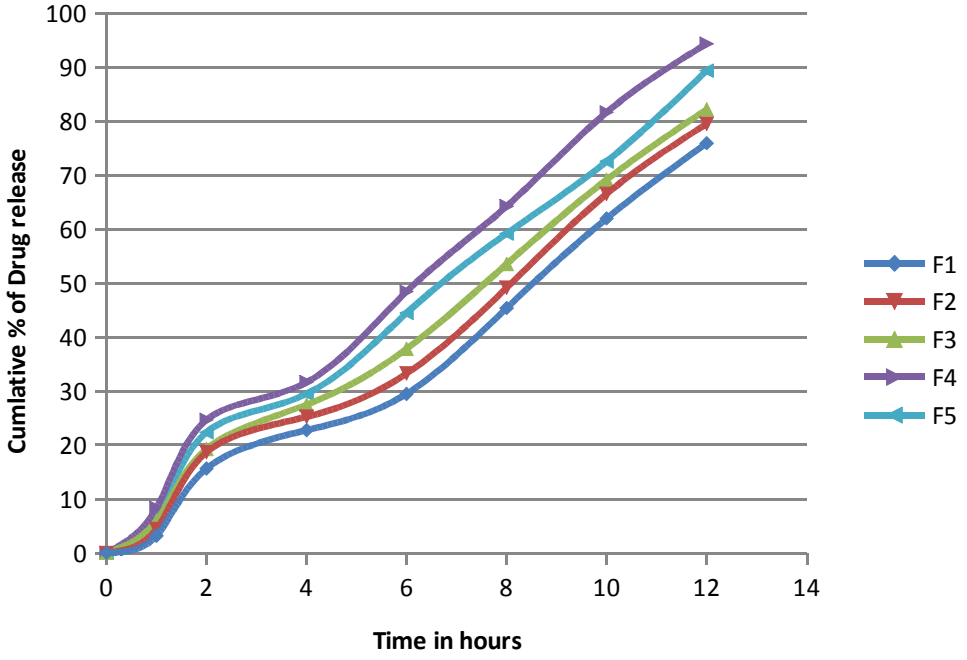
Dissolution profile of (F1-F5) formulation:

Table 20*: In vitro* drug release of Aceclofenac (F1-F5) in pH 7.4 phosphate buffer.

S.No	Time (hrs)	F1	F2	F3	F4	F5
		%drug released ± SD	%drug released ± SD	%drug released ± SD	%drug released ± SD	%drug released ± SD
1.	01	3.16 ± 0.056	4.41 ± 0.045	5.98 ± 0.063	8.32 ± 0.025	6.51 ± 0.045
2.	02	15.62 ± 0.22	18.63 ± 0.065	19.24 ± 0.045	24.63 ± 0.066	22.34 ± 0.099

3.	04	22.73 ± 0.45	25.21 ± 0.23	27.45 ± 0.45	31.62 ± 0.26	29.46 ± 0.45
4.	06	29.47 ± 0.54	33.23 ± 0.65	37.82 ± 0.52	48.41 ± 0.57	44.43 ± 0.35
5.	08	45.36 ± 0.35	49.12 ± 0.69	53.51 ± 0.52	64.23 ± 0.89	59.17 ± 0.48
6.	10	61.97 ± 0.66	66.52 ± 0.54	69.15 ± 0.63	81.62 ± 0.55	72.53 ± 0.63
7.	12	75.91 ± 0.62	79.48 ± 0.78	82.18 ± 0.36	94.32 ± 0.65	89.36 ± 0.85

Figure 16: *In vitro* drug release of Aceclofenac (F1 – F5):



Stability studies:

Table 21: Stability study of Aceclofenac formulation (F4) at 40±2°C/75±5%RH :

S.No.	Time in days	Physical changes	% Drug Content ± SD (40±2°C/75±5%RH)
1.	01	--	98.64 ± 0.82

2.	10	No change	98.00 ± 0.30
3.	20	No change	97.35 ± 0.51
4.	30	No change	97.09 ± 0.40

Table 22: ***In vitro % drug*** **Release of Aceclofenac (F4) after1month placing in stability chamber:**

S.No.	Time (hrs)	Cumulative % Drug Released ± SD at 40±2°C/75±5%RH	
		1st Day	30th Day
1.	01	8.32 ± 0.025	8.19 ± 0.20
2.	02	24.63 ± 0.066	23.34 ± 0.60
3.	04	31.62 ± 0.26	29.27 ± 0.22
4.	06	48.41 ± 0.57	47.35 ± 0.99
5.	08	64.23 ± 0.89	65.19 ± 0.81
6.	10	81.62 ± 0.55	80.26 ± 0.12
7.	12	94.32 ± 0.65	93.34 ± 0.24

DISCUSSION

The drug and the excipients compatibility studies shows there is no physical characteristic change shown in **Table -12**

The DSC trace of Aceclofenac shows five endothermic peaks at 153.96°C, 152.93°C. as shown in **Figure no : 6 - 8.** The first peak is associated with the melting of Aceclofenac (reported m.p is 149 - 153°C). The second peak showed no drastic changes from the melting point of the pure drug. The polymer did not show any characteristic endotherms. This is attributed to evaporation of absorbed water from the polymer during heating. So, Aceclofenac is chemically compatible with the polymers as shown in **Table-13.**

The IR spectrum of Pure Aceclofenac and its formulation (F4) were found to be similar to the peaks of both the spectrums are matching. IR studies have no interaction between drug and excipients as shown in **Figures 9 – 11.**

The apparent bulk density and tapped bulk density values ranges from 0.323 to 0.365 g/cc and 0.398 to 0.469 g/cc respectively. The results of angle of repose and compressibility index (%) ranges from 27.32 ±0.33 to 32.07±0.66 and 18.844 to 22.174 respectively. And the moisture content shows less than 1% in all formulations. The results of angle of repose (<35) and compressibility index (<23) indicates fair to passable flow properties of the powder mixture as shown in **table-14.**

The hardness of the tablet (F1-F5) was found to be in the range of 5 – 6 kg/cm^2 as shown in **Table-15**. The friability values were found to be in the range of 0.34 to 0.78% as shown in **Table-15.**

All the prepared tablets of Aceclofenac were evaluated for weight variation. The weight of all the tablets was found to be uniform with low values of standard deviation and within the prescribed IP limits of ±7.5% as shown in **Table-15.**

The low values of standard deviation includes uniform drug content within the tablets. The percent drug content of all the tablets was found to be in the range of 97.2 to 100.6 % as shown in **Table-15.**

The standard graph of Aceclofenac in pH 7.4 showed good linearity. The λ_{max} of Aceclofenac was determined in pH 1.2, pH 6.8, pH 7.4 and was found to be 276 nm.

The release of Aceclofenac from the different formulations was measured using UV-spectrophotometer. In pH 7.4 phosphate buffer formulation F-4 showed drug release of 94.32 % at the end of 12 hours has shown in **Table 20**. So formulation F4 has been selected as the best formulation and it was taken for stability studies.

Stability studies was carried on the best formulation F-4 at 40°C±2 / 75%±5 RH for 1month. It showed no significant changes in physical appearance, drug content and *in vitro* dissolution as shown in **Tables- 21-22.**

SUMMARY

In the present research work, the colon-targeting tablets of Aceclofenac were prepared by wet granulation. Formulation F1-F5 were formulated by taking drug-excipient in the ratio of Aceclofenac, MCC, Eudragit S100, Talc, Magnesium stearate.1:1, 1 : 0.16, 1 : 0.4, 1: 0.016, 1: 0.008 Respectively .The pure drug and the excipients were subjected to compatibility studies by DSC and FT-IR IR spectroscopic studies indicated that there are no drug-excipient interactions. DSC obtained showed no drug-polymer interaction.

All the tablets were subjected to pre- formulation evaluation studies by bulk density, tapped density, carr's index, hausner's ratio and angle of repose. The tablets are compressed and subjected to post formulation evaluation test.

Tablets prepared by wet granulation method were found to be good without any tabletting problems such as chipping, capping, sticking and lamination. Hardness, friability ,weight variation, thickness ,drug content uniformity were evaluated and found to be within the limits. The standard graph of pure drug Aceclofenac in pH 7.4 phosphate buffer was constructed and a linear graph was obtained.

From the *in vitro* dissolution studies carried in pH 7.4 phosphate buffer formulation F-4 was found to show retardation of drug release when compared F-1,F-2,F-3 and F-5 formulations. Hence F-4 was selected as the best formulation for colon drug delivery and it was subjected to stability studies.

Accelerated stability studies of F-4 formulation conducted according to ICH guidelines indicated that there are no significant changes in drug- content and *in vitro* dissolution release.

CONCLUSION

Aceclofenac is a non-steroidal anti- inflammatory drug and analgesic which used in the treatment of rheumatoid arthritis, Osteoarthritis, ulcerative colitis. Its usual dose is 100mg for 1 time. It causes gastric irritation when taken in morning. So an attempt has been done to formulate and evaluate Aceclofenac tablets to release the drug specifically to the colon by using a pH dependent polymer Eudragit S100 at pH 7.4 by wet granulation technique. This attempt was successful by formulation F-4 which was capable of releasing 94.32% in 12 hrs. Long-term stability studies have to be done for the formulation. *In vivo* studies have to be performed to conform the studies on Aceclofenac colon specific drug delivery systems

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